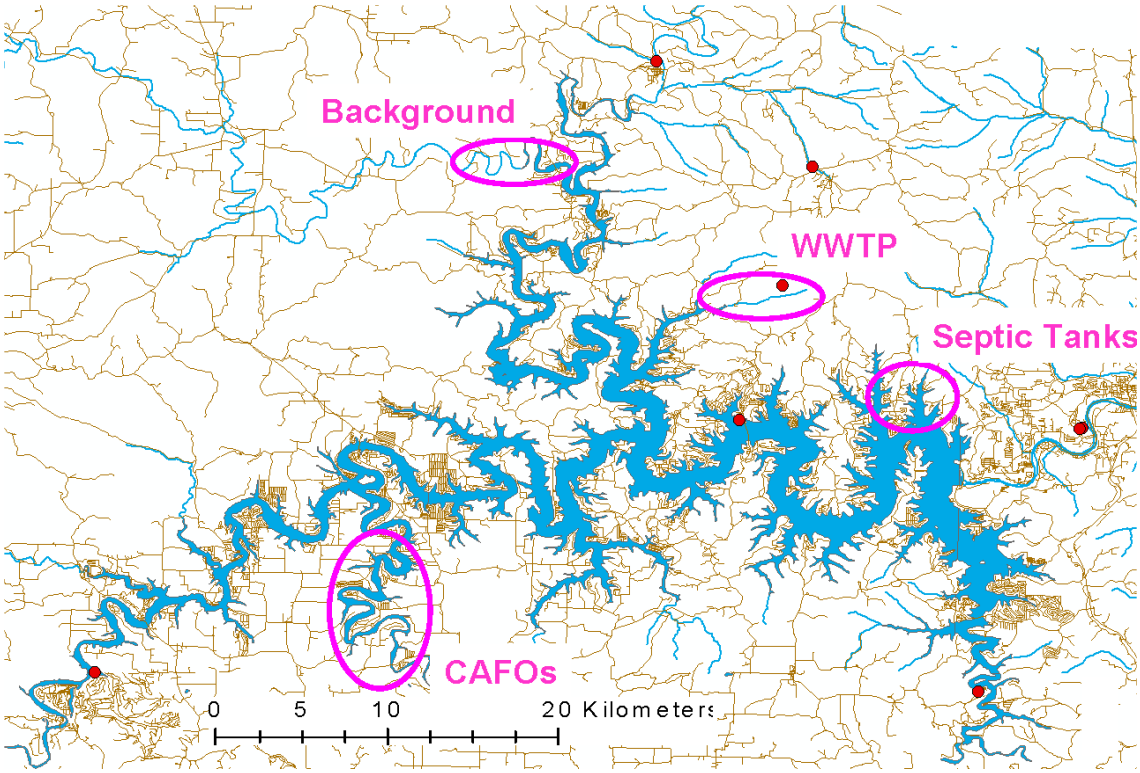
National Decentralized Water Resources Capacity Development Project



Evaluation of Chemical and Biological Indicators for Source Apportionment of Phosphorus in Table Rock Lake, on the Missouri-Arkansas Border

Submitted by Washington University
St. Louis, Missouri

February 2006

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ABSTRACT

Phosphorus contamination of surface waters from point and nonpoint sources remains an environmental problem of great concern. This project evaluated chemical and biological species as potential indicators of specific phosphorus source types. Evaluation of a suite of chemical and biological species was performed through field sampling and laboratory analysis. Samples were collected from potential sources and in the near-field of sources to determine whether the source profiles were apparent in the receiving water. This project was conducted at the Table Rock Lake watershed on the Missouri-Arkansas border. A geospatial information systems (GIS)-based multicriteria decision analysis was used to choose sampling locations in Table Rock Lake to capture the influence of discharges from wastewater treatment plants and septic systems and runoff from animal feeding operations (AFOs). A suite of chemical species was evaluated for potential indicators. The following are requirements of useful indicators:

- Presence in the receiving waters at detectable concentrations
- Uniqueness of source signatures
- Consistent concentration ratios of potential indicators to phosphorus

Almost all of the chemical species, except for synthetic organic compounds (SOCs), met the requirement of having detectable concentrations. Bromide was a unique indicator of large wastewater treatment plants (WWTPs). No other chemical species observed could be used as unique indicators of other sources. However, nickel and copper can potentially be used as indicators of septic system effluents. Sulfate can potentially be used as an indicator of WWTPs for receiving waters with larger proportions of water from these source types. No chemical species observed had consistent concentration ratios to phosphorus for all sources and seasons due to the high variation of phosphorus concentrations for the three septic systems and a small WWTP.

Coliphages were evaluated as potential biological indicators for wastewater input from human and nonhuman origins. A reverse transcriptase polymerase chain reaction (PCR) technique was used to identify bacteriophages, and traditional methods were used to quantify bacteriophages. This study shows that F^+ RNA phages can be used as biological indicators of fecal pollution; however, these phages cannot be used to distinguish between human and nonhuman sources because nonhuman bacteriophages were present in sources of human fecal pollution. Phages also cannot be used for phosphorus source apportionment because there was no statistically significant correlation between phage numbers and total phosphorus concentrations. Seasonal effects on bacteriophage presence were found, as winter samples contained the highest concentration of coliphages, while fall and spring samples contained the lowest.

EXECUTIVE SUMMARY

Phosphorus Pollution of Surface Waters and Table Rock Lake

Phosphorus contamination of surface waters from point and nonpoint sources remains an environmental problem of great concern. Excess loading of phosphorus to surface waters can cause eutrophication. The identification of sources and determination of their relative contributions to the total pollutant load are essential to achieving water quality goals. Source identification and apportionment are challenging due to the large number of potential sources and the significance of nonpoint sources.

This project evaluated chemical and biological species as potential indicators of specific phosphorus source types. Evaluation of a suite of chemical and biological species was performed through field sampling and laboratory analysis. Samples were collected from potential sources and at locations in the near-field of sources to determine whether the source profiles were apparent in the receiving water.

This project was conducted at the Table Rock Lake watershed on the Missouri-Arkansas border. Table Rock Lake is a reservoir that was created by a dam built by the US Army Corps of Engineers in 1958. The lake has a surface area of approximately 43,100 acres and the watershed upstream of the dam encompasses 4,020 square miles. Phosphorus concentrations in Table Rock Lake have increased over the past two decades, and previous studies reveal that phosphorus is the limiting nutrient in the lake. The lake is impacted by point sources (for example, municipal wastewater treatment plants) and nonpoint sources including decentralized wastewater treatment systems (for example, septic systems) and animal feeding operations.

Project Objectives

The project objective was to evaluate chemical and biological species that could potentially be used as indicators of specific types of phosphorus sources so they could be used in source apportionment methods. Due to the duration and size of the project, the objective was limited to evaluating potential indicators. This project did not encompass actual source apportionment. The hypothesis was that certain chemical and/or biological species would be found that met requirements of useful indicators. It was not known at the start of the project whether such species would be found.

Three specific objectives were pursued:

- Apply a multicriteria geospatial information systems (GIS) analysis to identify suitable sampling locations in Table Rock Lake impacted by single source types
- Evaluate a suite of chemical species (anions, major and trace elements, and synthetic organic compounds) to see if they were useful indicators for phosphorus source apportionment
- Evaluate selected bacteriophage species as biological indicators, as their presence—or absence—could act as an indicator of human versus nonhuman fecal contamination, which could correlate with sources of phosphorus

These three objectives were pursued in an integrated project involving geospatial data analysis, aquatic chemistry, and environmental microbiology. The GIS approach is described in Chapter 2. Chapters 3 and 4 are dedicated to the evaluation of chemical and biological indicators, respectively.

Multicriteria Approach to Selecting Sampling Sites

To ensure the development of representative source profiles, it was important to sample at locations impacted by a single source type. A GIS-based multicriteria decision analysis was applied to identify suitable sampling locations in Table Rock Lake to capture the influence of discharges from wastewater treatment plants (WWTPs) and septic systems, and runoff from animal feeding operations (AFOs). Sampling locations were also determined for background sites.

The GIS used for site selection was developed using data gathered from Missouri and Arkansas spatial data clearinghouses and environmental protection offices. GIS-multicriteria suitability analysis was determined based on characteristics of datasets (for example, soil depth in a soil type dataset) and distances from attributes (for example WWTP location). Data used in this analysis included:

- Topography
- Soil type
- Water bodies
- Roads
- Land cover
- Water discharge permit facility locations

The suitability analysis resulted in four site selection (suitability surface) maps, one for each of the siting criteria—WWTP, septic system, AFO, and background conditions. Creating the multicriteria model provided a systematic approach to site selection and a flexible and dynamic tool for future variations in the site selection process. The most suitable background sampling sites were identified on the James River arm in the northern branch of the lake.

The most suitable locations for isolating wastewater treatment plant effluent were in the central regions of the lake. The Kings River arm in the southwestern section of the lake stood out as most suitable for sampling animal feeding operation runoff. Indian Point in the eastern portion of the lake was identified as the most suitable area for sampling septic system effluent. The suitability maps were provided as guidance to the water quality sampling team who ultimately—based on personal lake experience and logistical considerations—chose the final sampling locations:

- The James River arm (background)
- The Branson West WWTP (centralized wastewater treatment)
- Water surrounding Indian Point (septic system influence)
- The Kings River arm (AFOs)

Evaluation of Chemical Indicators

A suite of chemical species was selected for evaluation for potential indicators. These species included:

- Anions (Br^-, Cl^-, F^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-})
- Major elements (Ca, Mg, K, Na)
- Dissolved and total trace elements (As, Ba, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Sb, Sr, U, V, Zn)
- Synthetic organic compounds (SOCs) (acetaminophen, caffeine, sulfamethoxazole, trimethoprim)

The utility of the selected species was evaluated for the following requirements of useful indicators:

- Presence in the receiving waters at detectable concentrations
- Uniqueness of source signatures
- Consistent concentration ratios of potential indicators to phosphorus

The potential indicators were not evaluated for requirements regarding transport and degradation, which are also important and can be evaluated in subsequent work focused on those species that meet the first three requirements.

Source profiles of various phosphorus sources within the Table Rock Lake watershed were examined through quarterly field sampling for one year. Samples were collected from potential sources, at locations in the near-field of sources, to determine whether the source profiles were apparent in the receiving water, at a background site not expected to be impacted by anthropogenic sources, and in public water supplies that are the influents to the wastewater treatment systems.

The concentrations of chemical species were determined using laboratory analysis following standard methods. The interpretation of the large dataset was supported by principal component analysis (PCA), a multivariate statistical method that examines the relationships within a large set of variables. PCA was used in an effort to establish source signatures—a unique combination of several species that are characteristics of a certain source—based on concentrations measured at source-rich receiving water locations.

Almost all of the chemical species met the requirement of detectable concentrations, with the exception of SOCs, as analyzed by high-performance liquid chromatography (HPLC) with ultraviolet detection. To measure trace-level SOCs present in environmental water samples, a mass spectrometric detector is required. Efforts were made to have SOCs analyzed by an outside laboratory with expertise in this area, but these efforts were ultimately unsuccessful because the receiving lab lost the samples.

Bromide (Br^-) is a unique indicator for the large WWTP, which was confirmed by PCA. No other chemical species were observed that could be used as unique indicators of other sources. However, nickel and copper can potentially be used as indicators of the septic system effluents, and sulfate as an indicator of WWTPs for receiving waters with larger proportions of water from these source types. SOCs might be more unique indicators than the other chemical species because human inputs are the main sources of SOCs, while the other species also have natural sources. Because of the large volume of the lake, smaller discharges from septic systems can be rapidly diluted with water from other locations. Consequently, the imprint of the source profiles on the receiving water is difficult to observe. In contrast, the effect of the larger WWTP on downstream river and lake sites can be observed by current analytical methods.

No chemical species were observed to have consistent concentration ratios to phosphorus for all sources and seasons, due to the high variability of phosphorus concentrations for the three septic systems and the smaller WWTP. However, some chemical species had consistent concentration ratios to phosphorus in a single source type (larger WWTP), and some species' concentrations (not ratios) were relatively constant throughout the whole year and may be used to apportion the contribution of water (not phosphorus) from various sources.

Evaluation of Coliphages as Biological Indicators

A suite of three RT-PCR primers specific for F^+ RNA coliphages was designed to discriminate between human and nonhuman fecal pollution. This method was tested with samples collected from Table Rock Lake. Sampling and onsite analyses were performed once per season to assess the effects of seasonal variation in source loadings and lake dynamics. Sampling locations and events for July 2004 through January 2005 were the same as those for chemical species. In addition, a subset of samples was also collected during May and August 2005. The RT-PCR technique was used to identify bacteriophages, and single agar layer (SAL), double agar layer (DAL), and traditional most probable number (MPN) assays were used to quantify the bacteriophages.

The study shows that F^+ RNA phages can be used as biological indicators for fecal pollution; however, the phages cannot be used to distinguish between human and nonhuman sources, nor for phosphorus source apportionment. Environmental samples from locations in the watershed that were most impacted by fecal pollution, as determined by the GIS approach, gave higher levels of F^+ RNA coliphages than the least impacted locations. However, results with genotyping did not show a correlation between the presence of human coliphages at expected human impacted locations and the presence of nonhuman coliphages at nonhuman impacted locations. Genotyping was not successful in determining the source of pollution, primarily because nonhuman bacteriophages were present in sources of human fecal pollution. There was no statistically significant correlation between phage numbers and total phosphorus concentrations.

The original experimental plan was designed to identify sources of human fecal pollution by enumeration and detection of phages specifically infecting *B. fragilis* HSP40. This target phage was chosen because in European studies, primers that amplified human-specific bacteriophages for *B. fragilis* showed them to be present in water that was impacted by human fecal pollution. However, the primers that targeted *B. fragilis* HSP40 phages in Europe were unable to detect *B. fragilis* HSP40 phages in the USA.

Seasonal effects on bacteriophage presence were found in this study. Winter samples contained the highest concentration of coliphages, while fall and spring samples contained the lowest. Samples from the summer showed higher F^+ coliphage concentrations than the spring and fall, but not as high as the winter. The propagated coliphages during the summer of 2005 were not amplified with the primers used in this study and remain an unknown strain(s). In the summer, a more resilient and unknown type of coliphage may have been present. Other factors besides seasonal fluctuations may have contributed to different coliphage numbers and types. For example, the hydraulics of the lake may have also contributed to the different types and concentrations of F^+ RNA coliphages over the seasons.

Summary of Recommendations for Future Work

After completing this 18-month project, insights were gained that can be useful in planning future studies. Several lessons from this project are summarized below, with respect to GIS-based multicriteria analysis, chemical indicator evaluation, and bacteriophage indicator evaluation.

Sampling site selection is ultimately determined by human knowledge and experience. Suitability analysis is a valuable tool in systematically providing information for multicriteria decision making. When combined with “local” or onsite information, it guides the final determination of sampling sites. The development of a GIS-multicriteria tool allows the site selection process to include feedback and adjust to new information as it becomes available in improving site selection. Analyzed samples can provide data on whether samples collected were actually influenced primarily by a single source type or if the location on the lake is impacted by other sources. This information can be assimilated into the suitability analysis to provide revised sampling sites for future sampling campaigns.

In the evaluation of chemical species, the inability of any species to meet all of the requirements of useful indicators was strongly affected by

- Variation of phosphorus concentrations in the sources
- Lack of species that were completely unique to a source type (except for bromide)
- Large size of the main body of the lake, which obscured the imprint of source profiles on the receiving lake waters

The four sampling campaigns of the current project were carried out in four different seasons in one year. The phosphorus concentrations of investigated source sites varied considerably, which made finding suitable indicators with constant indicator ratios impossible. Alternatively, if multiple sampling campaigns (and more sampling campaigns) for the source sites and receiving waters could be performed in a short period, the concentrations of phosphorus and other chemical species are less likely to vary as much in one year. This practice could help identify suitable indicators more easily because the distribution pattern of chemical species among sites would be more stable. However, these indicators may only be useful for short periods of time, and different seasons may have different indicators due to seasonal variation in the composition of sources. In addition, the more frequently the sites are sampled, the more accurate the PCA interpretation is as a statistical tool.

SOCs might be better indicators with respect to uniqueness because human inputs are the main sources of certain SOCs. Collections of septic systems may have discharge volumes that are too small compared to the large lake water body and their effects on the water quality of receiving waters are difficult to observe. Several indicators would have been evaluated more favorably for a smaller lake with similar source magnitudes.

Additional studies are needed to elucidate the diversity of the coliphage population in the Table Rock Lake watershed. These studies could shed light on coliphages that could be used as an indicator of phosphorus pollution. To ascertain which coliphages are present in the watershed, all 18 known F^+ RNA coliphages in the four subgroups must be targeted instead of just the three coliphages used in this study. Thus, an additional 15 primer sets must be developed and tested.

In terms of methodology, development of a direct quantitative polymerase chain reaction (qPCR) assay of filtered environmental samples should have high priority. Such an assay would both identify and quantify the phages present, while eliminating the propagation step. This is advantageous because propagation adds time and effort, introduces the potential for contamination, and may have a lower sensitivity compared to qPCR.

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1 INTRODUCTION AND BACKGROUND

1.1 Introduction

Phosphorus concentrations in Table Rock Lake in Southwestern Missouri have increased in recent years (Thorpe *et al.* 2004). In 2002, the Missouri Clean Water Commission placed Table Rock Lake on its list of impaired waters because of high phosphorus concentrations (Missouri Department of Natural Resources 2003). Development and population growth associated with the lake's status as a recreation and tourist destination and agricultural activities in the watershed are the primary causes of the changes in phosphorus concentrations. The increase has raised concerns over the potential for eutrophication, a process in which waters become choked with algal blooms and deep waters develop hypoxic conditions that are lethal to fish and other aquatic organisms (Tchobanoglous and Schroeder 1985). The increased levels of phosphorus indicate an initial negative impact on the lake's water quality and ecosystem health, and a subsequent negative impact on the local economy dependent on tourism. The lake is impacted by a variety of sources, including point sources—for example, municipal wastewater treatment plants (WTTPs)—and nonpoint sources, such as decentralized wastewater treatment systems (for example, septic systems), confined animal feeding operations (AFOs), poultry litter applied to farm fields, and storm water runoff. The identification of sources and determination of their relative contribution to the total pollutant load are essential to achieving water quality goals, but source identification and source apportionment are challenging due to the large number of potential sources and the significance of nonpoint sources.

Point source and nonpoint source (NPS) phosphorus contamination of water estuaries remains an environmental problem of great concern. Human population growth, inadequate sanitation, and mismanagement of animal waste contribute to increasing fecal pollution of both surface and undergroundwater resources. Environmental engineers have been successful in curbing pollution from point sources, such as domestic and industrial sources, since the 1972 Clean Water Act and amendments in 1977 and 1987 were enacted and enforced (Field *et al.* 2003; US EPA 1986).

NPS pollution has been harder to control because it occurs mainly through dispersed storm water runoff from farmland, city streets, construction sites, suburban lawns, roofs, and driveways. This runoff often contains harmful substances including toxic compounds, excess nutrients, and sediments (Foy *et al.* 2001; Lennox *et al.* 1998). Nutrients from point sources and NPSs play a major role in eutrophication in lakes and ponds because small amounts of phosphorus result in excessive aquatic weed and algae growth. Subsequent plant biomass decay causes depletion of oxygen, and thus an almost complete elimination of aquatic life. To curb phosphorus input, and therefore eutrophication, the human and animal origin of pollution must be known (Grabow 1996; Sinton *et al.* 1998)

Different indicators were evaluated specifically for Table Rock Lake that would be required to perform a source identification and source apportionment approach to phosphorus pollution. The work included sampling events and analytical approaches to test several biological and chemical indicators. The work presented here is divided into three different chapters. To determine the sampling locations, a scientific approach was used that included a multicriteria geospatial information systems (GIS) approach. This approach is described in Chapter 2. From this work, a seasonal sampling plan was developed. Samples taken from the Table Rock Lake watershed were used to test the indicators. Chapter 3 and Chapter 4 are dedicated to the evaluation of chemical and biological indicators, respectively. The interpretation of the dataset of chemical species was augmented by factor analysis, a multivariate statistical analysis tool. Chapters 2, 3, and 4 are divided into the following sections:

- Introduction
- Methods and Materials
- Results
- Discussion
- Summary and Recommendations for Future Work

1.2 Background

The following sections explain phosphorus pollution and the need for and methods of finding its sources.

1.2.1 The Challenge of Phosphorus Pollution in Lakes

Phosphorus is an essential nutrient to almost all forms of life, but excess amounts of phosphorus can be detrimental and even disastrous to aquatic systems. High nutrient loading rates to surface waters have led to excessive biological growth, a process known as eutrophication. The rate of algal growth is controlled by the concentration of the limiting nutrient. The ratio of nitrogen to phosphorus in algal biomass is 16:1, the Redfield ratio, and either nitrogen or phosphorus may be the limiting nutrient.

Nitrogen, as a nutrient, is generally present in lakes as nitrate, and phosphorus as a phosphate. Phosphorus is often the rate-limiting nutrient, because nitrogen can be taken up from the atmosphere as nitrogen gas through microbiological processes. Phosphorus loads in surface waters are distributed between dissolved forms and forms bound to suspended solids, and biogeochemical processes of phytoplankton growth and decay cycle phosphorus through organic and inorganic species (Figure 1-1) (Schlesinger 1997). Concerns over eutrophication have led to the removal of phosphates from detergents and continues to drive interest in developing water quality criteria for phosphorus.

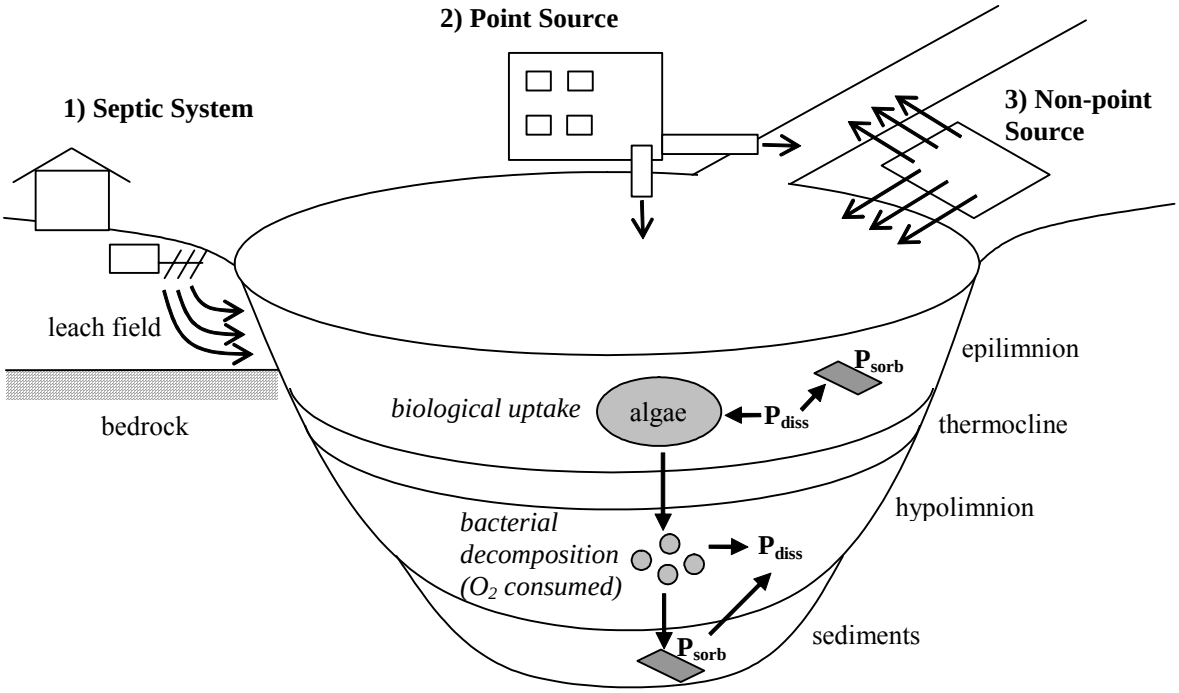

Figure 1-1
Schematic Representation of Point and Nonpoint Sources of Phosphorus to a Lake and a Simplified Representation of Phosphorus Biogeochemical Cycling

Phosphorus is introduced to surface waters from a wide variety of point and nonpoint sources (Figure 1-1). Point sources include municipal wastewater discharges and permitted industrial and agricultural waste streams. By their nature, point sources can be easily monitored, and pollutant discharges from point sources can be controlled by the construction of engineered treatment systems.

The determination and control of pollutant fluxes from nonpoint sources are substantially more challenging. Nonpoint sources of phosphorus include urban and agricultural runoff and effluents from decentralized wastewater treatment systems.

Decentralized wastewater treatment systems can be characterized as a large collection of point sources that, due to their diffuse distribution, may also be viewed as nonpoint sources. Domestic wastewater entering septic systems is rich in nitrogen and phosphorus. When phosphate is mobile in the leachfields of septic systems, these systems can be important nonpoint sources of phosphorus to adjacent surface water bodies. For properly designed and functioning septic systems, significant amounts of nitrate are released, but phosphate is immobilized in the leachfield by binding to porous media (Wilhelm *et al.* 1994). However, phosphate release can occur if effluents are discharged to highly fractured porous media or to thin or sandy soils with limited capacity for phosphate binding to mineral-surfaces. Phosphate can be mobile in septic system leachfields if the soil and water chemistry are not favorable for phosphate binding (for example, high pH or low abundance of iron and aluminum oxyhydroxides) or if the binding capacity of the available porous media becomes saturated (Robertson *et al.* 1998).

At Higgins Lake in Michigan, high water tables and sandy soils with limited phosphorus binding capacities contributed to the failure of septic systems to limit phosphorus release to adjacent surface waters (Minnerick 2001).

At Table Rock Lake on the Missouri-Arkansas border, malfunctioning or nonfunctioning septic systems have been identified as sources of phosphorus to the lake (Table Rock Lake Water Quality 2003). The failure of septic systems is attributed to the highly fractured karst subsurface geology of much of the watershed and to thin soils that provide little treatment of phosphate in the leachfield (Midwest Environmental Consultants 2001). Important sources of phosphorus from runoff include AFOs, fertilized lawns, and cropped fields (Hooda *et al.* 2000; Penn and Sims 2002; Westra *et al.* 2002).

At Friary Lough in Northern Ireland, significant inputs from agricultural nonpoint sources caused phosphorus concentrations in the lake to continue increasing even after installation of treatment processes to limit phosphorus release from centralized wastewater treatment plants (Foy *et al.* 1995). Phosphorus increases in Friary Lough have been explained by the influx of phosphorus from soils whose binding capacities have been saturated, and much of the flux from agricultural fields has come through the groundwater (Jordan and Rippey 2003; Jordan *et al.* 2001).

In a more positive recent result, actual phosphorus concentrations in rivers of heavily agricultural Northwest Ohio were lower than estimated by a phosphorus budget approach because of improved management of NPSs (Baker and Richards 2002). Phosphorus release, following application of inorganic or manure fertilizers to fields, depends upon the specific site hydrology and soil type (Gburek *et al.* 2000).

1.2.2 Need for Source Apportionment

The Clean Water Act introduced the total maximum daily load (TMDL) approach for achieving desired water quality criteria. In a TMDL plan, point and nonpoint sources must be controlled to meet an acceptable total daily load to the receiving water body from all sources. The identification of phosphorus sources and determination of their relative contributions to the total nutrient load are essential to achieving water quality goals, but source tracking and source apportionment are challenging due to the large number of potential sources and the significance of NPSs.

Source tracking involves identifying the specific source responsible for a given pollutant, and source apportionment involves determining the relative contributions of multiple sources that contribute to the occurrence of a given pollutant. Source tracking methods have most commonly been applied to the determination of sources of pathogenic organisms, which may reasonably be considered to originate from a single source. The diversity of sources of phosphorus to lakes makes it necessary to take a source apportionment approach that can account for a range of point and nonpoint sources. The identification of chemical and biological species that can be used as indicators for phosphorus is crucial to the development of phosphorus source apportionment methods.

1.2.3 Water Quality Models Based on Land Use and Receptor Modeling

Established methods for estimating the contributions of pollutants from multiple sources are based on measurements of known point sources and estimates based on land use distributions for nonpoint sources. Water quality modeling methods based on land use include the loading functions developed by the US Environmental Protection Agency (US EPA) and regression equations developed by the US Geological Survey (USGS) (Wurbs and James 2002).

Loading functions calculate mass loads from predicted dissolved concentrations, runoff depths, and a coefficient to account for the proportion of the dissolved load in the runoff that reaches the surface water.

The USGS regression equations are empirical relationships that express pollutant loads as functions of parameters including:

- Total rainfall
- Impervious area
- Storm duration
- Mean annual rainfall
- Drainage area
- Land use percentages
- Population density

Regression equations have been developed for both dissolved phosphorus and total phosphorus.

The National Resources Conservation Service created the Phosphorus Index as a tool for estimating phosphorus export from agricultural watersheds based on information on the site hydrology and source characteristics; however, a recent study suggested modifying the index based on data that indicate that phosphorus export is related more to near-stream sources than to the phosphorus content of the whole watershed (Gburek *et al.* 2000).

Tools based on land use are useful for estimating effects on surface water quality, but these methods have limitations. Estimates of the contributions from nonpoint sources are only as good as the land use information available, which will likely not include total phosphorus application rates. As noted previously, the phosphorus index may require modification and the USGS regression equations are purely empirical.

A recent investigation of the water quality of Higgins Lake in Michigan illustrates the need for multiple approaches to determining sources of pollutants to lakes. Detailed land use information on building and road density showed a clear relationship to the chloride concentrations and turbidity at different locations in the lake, but phosphorus concentrations were not directly correlated with land use information. The measurement of *Escherichia coli* concentrations in the water was ultimately necessary to distinguish between phosphorus from septic systems and from lawn fertilizers (Minnerick 2001).

Receptor modeling is a potential alternative source apportionment approach to land use-based methods. In receptor modeling, concentrations of multiple species are monitored at a receptor location and then apportioned among different sources by comparing the chemical compositions of different sources. Key steps in receptor modeling are establishing the profiles, or "fingerprints" of specific source types and verifying the imprint of these profiles at downwind or downstream locations. Receptor modeling is widely used in the air quality field (Friedlander 1973; Hopke 1985), but it is rarely used for water quality modeling because the signatures of pollutant sources tend to change during transport. However, receptor modeling is a prospective method that may complement land use-based water quality modeling.

The success of phosphorus source apportionment using receptor modeling depends on the selection of an appropriate set of identifying indicator species. The term species is used here to refer to both chemical and biological constituents. The use of *E. coli* measurements at Higgins Lake to identify failing septic systems as the source of phosphorus is an example of the use of a biological indicator for source tracking of phosphorus inputs (Minnerick 2001). Recent work demonstrated the use of a large suite of organic chemicals as indicators for inputs from wastewater treatment plants (Glassmeyer *et al.* 2005). Other species, such as chloride and bromide, are conservative chemical indicators that can be used to study overall water transport and mixing (Effler *et al.* 2002; Peterson *et al.* 2004).

2 MULTICRITERIA APPROACH TO SELECTING SAMPLING SITES MONITOR SOURCE SPECIFIC IMPACTS

2.1 Introduction

Previous studies have modeled source contributions of pollutant loads in watersheds using export coefficients for land use types, topography, and geology (Endreny and Wood 2003; McFarland and Hauck 2001) or mass balance models (Pieterse *et al.* 2003). These methods sample and characterize the source and, based on transport and transformation properties, model the process to estimate downstream pollutant concentrations.

Another approach to source apportionment is through the identification of source profiles. This approach is successfully used in the air quality field. To establish source signatures, or "fingerprints," it is important to sample at the source, but to look for the influence of these profiles at downwind or downstream receptor locations. This overall study examined source profiles in Table Rock Lake with the objective of identifying chemical and biological indicators of phosphorus sources. To ensure the development of representative source profiles, it was important to sample at locations impacted by a single source. The objective of the work presented in this chapter was to apply geospatial information systems (GIS)-based analysis to identify suitable sampling locations in Table Rock Lake that were impacted by single source types.

Locating the "best" locations for sampling in support of an environmental field study can reduce project costs and improve the study results. The objectives of this project were similar to the classic GIS site selection analysis—using GIS thematic layers and site criteria and then determining locations that optimally met a set of siting criteria. GIS suitability analysis combines those requirements to identify areas that meet all criteria. In this study, the primary siting criterion was a location most likely impacted by a single phosphorus source type.

2.2 Background

These sections describe Table Rock Lake and explain multicriteria decision analysis using GIS as it was used in this study.

2.2.1 Table Rock Lake

In August of 2002, the Missouri Clean Water Commission placed Table Rock Lake, a large reservoir (41,300 acres) in Southwest Missouri, on the Missouri 303(d) list of impaired waters because of high phosphorus concentrations (Figure 2-1).

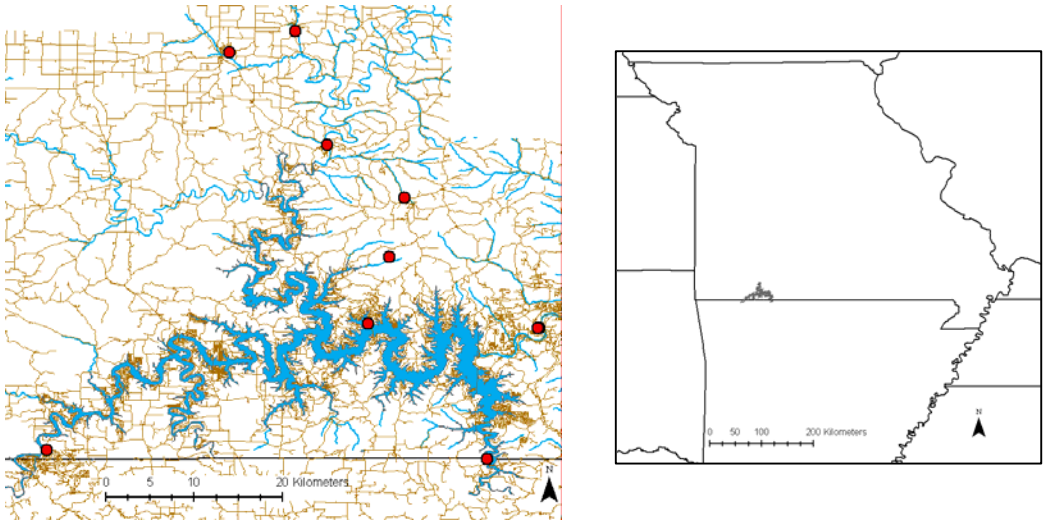

Figure 2-1
Table Rock Lake in Southwestern Missouri and Northwestern Arkansas[1]

Land use in the Table Rock Lake watershed (4,020 square miles) has been changing rapidly with population growth and increasing agricultural activities. The Missouri Department of Natural Resources estimated in 1998 that Table Rock Lake received about 2,200 pounds of phosphorus per day, one third from point sources and two thirds from nonpoint sources. Since 1998, the Lakes of Missouri Volunteer Program has monitored Table Rock Lake approximately monthly for nitrogen, phosphorus, chlorophyll, and Secchi transparency.

Table Rock Lake is characterized as a narrow lake with many arms. Soils near the lake tend to be thin and underlain with limestone bedrock.

2.2.2 Multicriteria Decision Analysis for Environmental Engineers

Multicriteria decision making is defined as choosing among alternatives where the alternatives are based on a set of evaluation criteria (Ascough *et al.* 2002; Eastman 1999; Malczewski 1999). The criteria can include a set of objectives, such as those required for deciding among policy

[1] Wastewater treatment plants are represented by dots; streams, rivers, and the lake are blue; roads are presented as brown lines.

scenarios, or characteristics of geospatial data, such as those used in determining the optimal location for an activity.

Geospatial information systems (GIS) software for spatial data processing, analysis and visualization, and its associated science are becoming common tools in river and lake water quality assessment studies. Advances in computer processing speeds and data storage and dissemination allow efficient handling and integration of large data sets, making GIS-type analyses commonplace in environmental engineering and sciences. Multicriteria decision analysis using GIS commonly applies a set of weight factors to each objective or data characteristic that are combined to derive the locations meeting the decision criteria. In GIS, this type of analysis involves the combination of mapped datasets and is commonly referred to as suitability analysis or weights of evidence.

Most sampling design methods are based on optimizing samples over space or time (Sanders *et al.* 1983). These methods are commonly used to design a long-term network of sampling sites, where the entire network has a single sampling objective. However, when individual samples are required for unique objects such as single source types impacting water quality, stratified sampling and general sampling design are not applicable. The work presented here applied GIS-based multicriteria decision analysis to the determination of sampling sites within a lake. Previous studies have used multicriteria analysis to investigate septic system sites (Stark *et al.* 1999), model pollutant levels in lakes (Endreny and Wood 2003), and river sampling network design (Dixon *et al.* 1999; Ning and Chang 2002).

2.3 Methods

These sections describe the methods and calculations used to choose the sites for this study.

2.3.1 Data and Software

The GIS used for site selection was developed using data gathered from Missouri and Arkansas spatial data clearinghouses and environmental protection offices. A GIS-based suitability analysis based on characteristics and distances from attributes of these datasets resulted in the identification of possible sampling areas for capturing the influence of discharges from wastewater treatment plants and septic tanks and runoff from AFOs. Sampling locations were also determined for background sites. GIS multicriteria suitability analysis was determined based on characteristics of datasets (for example, soil depth in a soil type dataset) and distances from attributes (for example wastewater treatment plant location and discharge flow rate in a permitted wastewater discharge facility dataset). Data used in this analysis included topography, soil type, water bodies, roads, land cover, and water discharge permit facility locations (Table 2-1).

Table 2-1
Dataset Used in Site Selection Analysis

Dataset Name	Data Content	Data Source
Missouri GIS Thematic Layers	Roads, Lakes, Rivers, Streams, Metropolitan Areas	Missouri Spatial Data Information Service
Arkansas GIS Thematic Layers	Roads, Lakes, Rivers	Arkansas GeoStor
Soil Survey	For Barry, Stone, and Taney Counties in Missouri and Carroll and Boone in Arkansas	USDA-NRCS Soil Survey Geographic Database
Elevation	Digital Elevation Model (DEM) Data Southwestern Missouri and Northwestern Arkansas	USGS National Map
MO Permitted Facilities	Permitted Wastewater Discharge Facilities	Missouri Department of Natural Resources, Water Protection and Soil Conservation Division
AR Permitted Facilities	Permitted Wastewater Discharge Facilities	Arkansas Department of Environmental Quality, Permit Data System

Some of the gathered datasets were processed to derive more relevant parameters for the site identification analysis. For example, the soil type dataset was filtered to create a subset that only included those soils characterized by depths less than 36 inches. Shallow soils are unsuitable for septic tank leaching fields and are likely to be areas with above average discharge to the lake. Urbanized areas were estimated by calculating road density based on the road network. The coupling of soil depth and road density was used to identify areas with a high probability of septic tanks located in shallow soils. The GIS analysis was conducted within the ESRI ArcGIS 8.3 software environment (ArcView 8.3 with Spatial Analyst Extension) (ESRI 2002).

2.3.2 Site Selection

Multicriteria GIS analyses were conducted to identify potential sampling locations in Table Rock Lake that were primarily impacted by:

- Wastewater treatment plant effluent
- Septic tank discharge
- Runoff from animal feeding operations
- No major anthropogenic source (background site)

Each siting requirement involved a separate suitability analysis, although in some cases the analyses shared common datasets. Figure 2-2 summarizes the data processing and GIS data layer weighting in deriving a sampling site suitability surface.

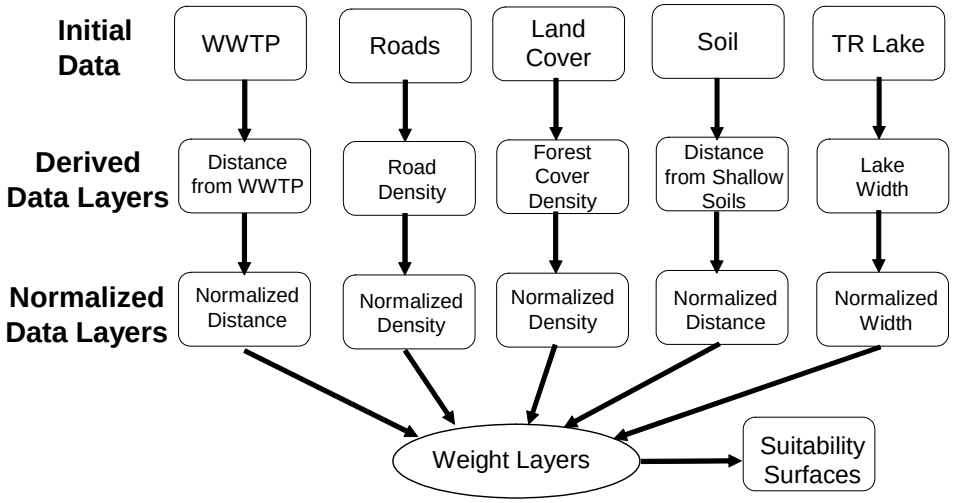

Figure 2-2
Data Flow for Suitability Surface Estimation

2.3.3 Calculation of Septic Tank Suitability Surface

Septic tanks are a significant source of phosphorus in Table Rock Lake (Midwest Environmental Consultants 2001). Municipal wastewater treatment plants do not service many residential and commercially zoned areas near the lake. The geology of the Table Rock Lake watershed, with thin soils and permeable subsurfaces, is not conducive to the effective performance of septic tank leachfields and can result in inadequately treated effluent entering the lake. Combining knowledge of septic tank locations with areas of thin soil and permeable geology provides an indicator for areas with a high probability of impacting phosphorus concentrations in Table Rock Lake.

2.3.3.1 Soil Depth

Soil data were acquired from the USDA-NRCS Soil Survey Geographic Database. The database provides soil type, soil depth, and other soil properties. A subset of the data was created by filtering the soil type dataset to include only those soils with a restrictive layer within 36 inches of the surface. To create a relevant metric to cover the area over Table Rock Lake, the shortest distance in the lake to the filtered soil types was calculated. To calculate the GIS site analysis weight factors, the distance values are normalized by dividing their frequency distribution into nine intervals based on standard deviations and assigning integer values of one through nine to the intervals. A weight value of nine represents areas on the lake that are near a shallow soil type and therefore are given more weight in the septic tank sampling location calculation (Figure 2-3).

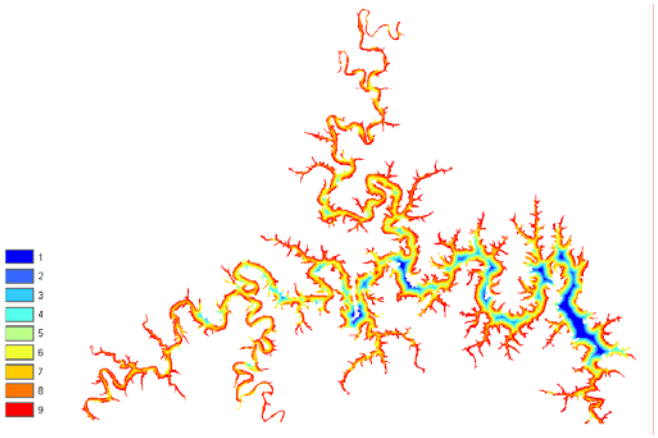

Figure 2-3
Map Algebra Calculation of the Distance to Shallow Soil Depth[2]

2.3.3.2 Septic Tank Density

Data on the number and locations of septic tanks around Table Rock Lake were not available for this study. However, areas with high population densities are representative of areas with high septic tank densities. Population data at a suitable spatial resolution were not available, and therefore road density was calculated as a surrogate for septic tank density.

A road density surface was calculated using road network data from the Missouri Spatial Data Information Service in a standard GIS feature density analysis tool. The tool calculates density for a location by identifying roads within a specified search radius around that location. A road density was calculated for locations both over land and in the lake.

In reality, the road density over most parts of Table Rock Lake is zero (the exceptions occurring near bridges). For this study, the road density value in the lake is meaningful because it indicates areas in the lake adjacent to residential neighborhoods. The road density values were classified into nine intervals based on the standard deviations of their frequency distributions. A classification of nine represented the highest road density, and therefore the highest estimated probability of septic tank density (Figure 2-4).

[2] Red and orange areas are the most suitable for sampling septic tank effluent. The units are based on classification into nine intervals based on the standard deviation of the frequency distribution of the given factor.

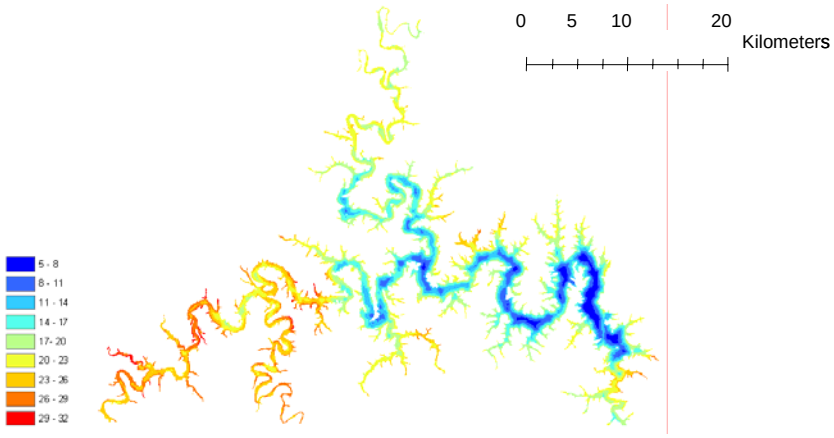

Figure 2-4
Map Algebra Calculation of the Nearby Road Density[3]

2.3.3.3 Wastewater Treatment Plants

In an effort to identify areas in the lake predominately impacted by septic system discharge, a third criterion was included to account for wastewater treatment plant (WWTP) locations. The distance for wastewater treatment plants was calculated along the path of streams and the lake to create a map surface of distance that was assumed to be inversely proportional to the likelihood of WWTP impact (the greater the distance from a WWTP, the lower the probability of WWTP impact). The WWTP distances were normalized to an integer range of one through nine based on the standard deviation of their frequency distributions (Figure 2-5).

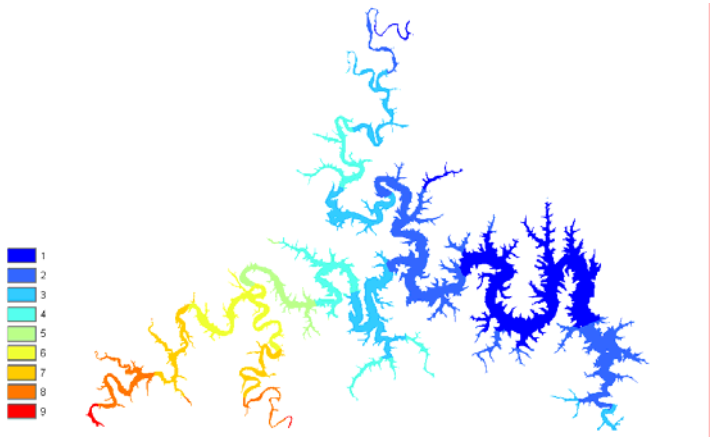

Figure 2-5
Map Algebra Calculation of the Shortest Distance to a Wastewater Treatment Plant[3]

[3] Red and orange areas are the most suitable for sampling septic tank effluent. The units are based on classification into nine intervals based on the standard deviation of the frequency distribution of the given factor.

2.3.3.4 Lake Width

A fourth factor included in the site suitability analysis was the width of the lake. The topographic features around Table Rock Lake form narrow and shallow channels. These "fingers" of the lake are also characterized as being some of the furthest from WWTPs, with the highest septic tank densities and thinnest soils. Therefore, the lake's fingers would be identified as ideal sampling locations by the site analysis. However, these shallow, narrow areas of the lake are difficult to reach by boat and for logistical reasons were de-emphasized in the site analysis. Since lake depth data were not available, a metric related to the width of the lake was used to represent these shallow channels in the site analysis.

The "width" was determined by calculating the distance in the lake from the Table Rock Lake shoreline and then the highest value within a 500-meter radius was used to represent the lake "width." As a site analysis weight, the lake width values were divided into nine intervals based on the standard deviations (Figure 2-6).

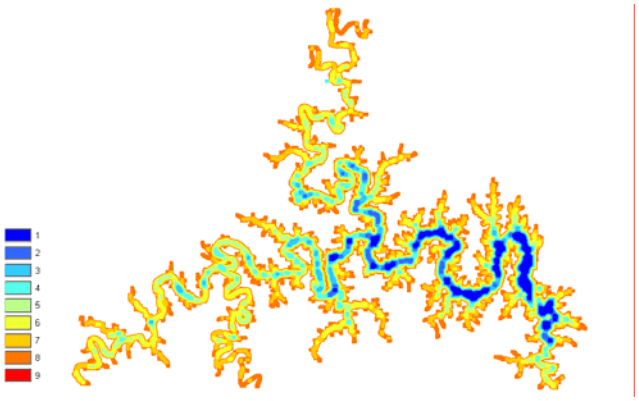

Figure 2-6
Map Algebra Calculation of the Lake Width[4]

2.3.3.5 Septic Tank Suitability Surface

The four factors were weighted equally in the calculation of the septic tank suitability surface (Figure 2-7):

Suitability Surface = Soil Depth + WWTP Distance + Septic Tank Density + Lake Width

[4] Red and orange areas are the most suitable for sampling septic tank effluent. The units are based on classification into nine intervals based on the standard deviation of the frequency distribution of the given factor.

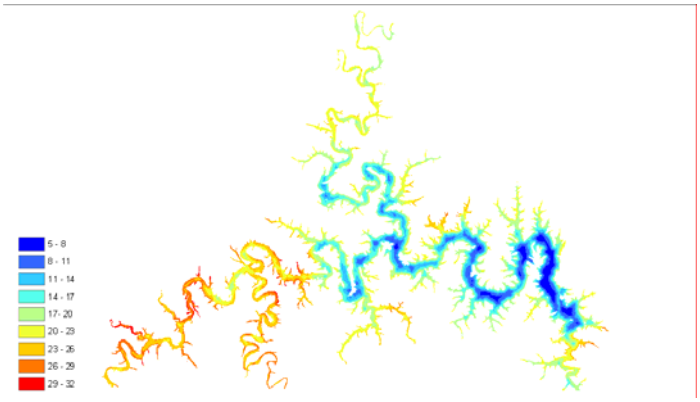

Figure 2-7
Map Algebra Calculation of the Septic Tank Suitability Surface[5]

2.3.4 Calculation of Areas Impacted Primarily by Wastewater Treatment Plant Effluent

The areas most likely impacted by only a wastewater treatment plant were determined by the shortest downstream distance from the nearest plant, while accounting for proximity to areas with high septic tank density. The most suitable locations for sampling wastewater treatment plants were assumed to be near a WWTP in areas with a low probability of being impacted by septic tank runoff.

2.3.4.1 Wastewater Treatment Plant Distance

The distance downstream of a wastewater treatment plant was calculated using features within the GIS software. Calculation of a distance within the lake (such as following the contour of the lake and not simply a straight line) required some data processing within the GIS environment. To calculate the downstream distance for every point in a stream or lake, lakes' and streams' polygon files (in shape-file format) were converted to raster formats. The raster format allowed the derivation of a binary raster with values of 1 indicating a stream or lake and values of 0 indicating non-stream, or non-lake. The "CostDistance" function in ESRI ArcGIS was used to calculate a distance (in units of raster cells) along the paths created by the binary grid, thereby providing a relative distance from the nearest wastewater treatment plant at each location along Table Rock Lake and its feeding streams (see Figure 2-5).

2.3.4.2 Septic Tank Density and WWTP Suitability Surface

The septic tank density was determined as was described in Section 2.3.3.2 and illustrated in Figure 2-4.

[5] Red and orange areas are the most suitable for sampling septic tank effluent. The units are the sum of the values from Figure 2-4 through Figure 2-6.

The final suitability surface for sampling wastewater treatment plant effluent was calculated by equally weighting the WWTP distance and septic tank density (Figure 2-4).

2.3.5 Calculation of Areas Impacted by Runoff From Animal Feeding Operations

The method applied in determining optimal locations for sampling the impact of runoff from commercial AFOs was similar to that for wastewater treatment plant effluent described in the previous section. The most suitable locations for sampling the influence of animal feeding operations were considered to be near high densities of animal feeding operations with a low probability of being impacted by WWTP or septic system discharges. The final suitability surface for sampling animal feeding operation runoff was calculated by equally weighting the CAFO distance, the distance from the nearest WWTP, and septic tank impact areas.

2.3.6 Calculation of Areas Least Impacted by Phosphorus Pollution

A sampling site that could record background conditions absent of the influence of potential phosphorus sources served as a control for comparing the source-impacted samples. Determining areas on the lake that could potentially serve as background sites required information on land cover/land use, wastewater treatment plants, and septic tank density. The ideal background site would be situated near forested areas remote from the influence of any wastewater treatment activities or other sources of phosphorus.

2.3.6.1 Weighting Factors for Area With Background Lake Conditions

The University of Missouri compiled a land cover dataset for the year 2000 from LandSat satellite imagery and aerial photographs. The land cover dataset classifies surfaces into 15 categories ranging from impervious and urban to forest and water. A background site was identified as being near surfaces classified as:

- Deciduous forest (forest with greater than 60% cover of deciduous trees)
- Evergreen forest (forest with greater than 60% cover of evergreen trees)
- Mixed forest (forest with greater than 60% cover of a mixture of deciduous and evergreen trees)

Areas of Table Rock Lake near the forested surfaces were determined by assigning a count of the number of grid cells within a 30 × 30 grid cell area that were classified as forested. The normalized count was used as the site analysis weight factor.

The GIS weighting factor used in accounting for areas of high septic tank density was the same as that used in the derivation of the septic tank's suitability surface (Figure 2-5). The final suitability surface for sampling background conditions was calculated by equally weighting the land cover type, WWTP distance, and septic tank density factors.

2.4 Results

The suitability analysis resulted in four site selection (suitability surface) maps, one for each of the siting criteria:

- Wastewater treatment plant
- Septic tank
- AFOs
- Background conditions

Some of the results were intuitive. Looking at a map with land cover, roads, and wastewater treatment plants could reveal suitable background sampling sites as those high in forest cover and absent of roads or wastewater treatment plants. However, creating a multicriteria model for identifying septic tank source sampling provides a systematic approach to site selection and a flexible and dynamic tool for future variations in the site selection process. The resulting maps derived from this analysis weighted all factors equally. The areas identified for each sampling type are shown in Figure 2-8.

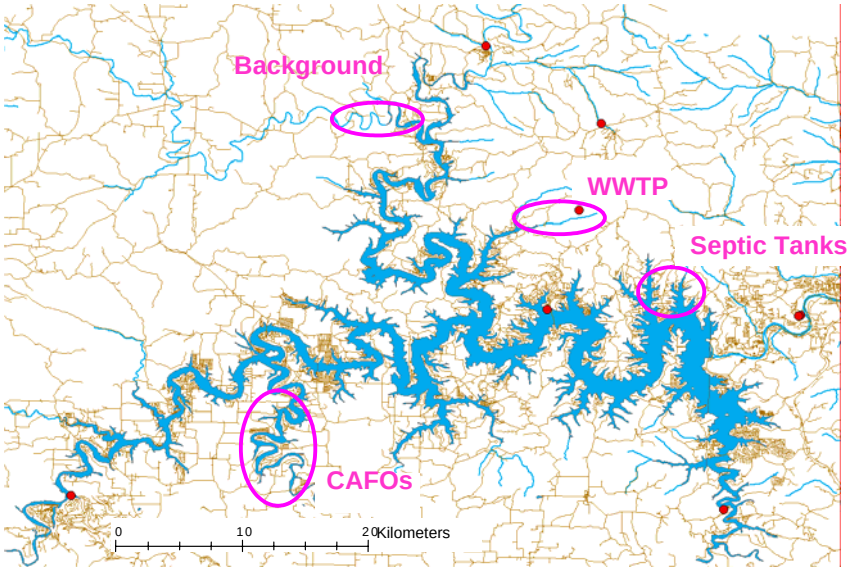

Figure 2-8
Locations Identified as Most Suitable for Sampling Table Rock Lake Impacted by WWTP, Septic System Discharge, Animal Waste Runoff, and Background Conditions

The most suitable background sampling sites were identified on the James River arm in the northern branch of Table Rock Lake. The most suitable locations for isolating wastewater treatment plant effluent were in the central regions of the lake. A number of locations—primarily near towns—were identified as highly suitable for sampling septic tanks. The area that stood out on the suitability surface map was near the town of Shell Knob on the western portion of the lake. The Kings River arm in the southwestern section of the lake was the most suitable for sampling animal feeding operation runoff. The suitability maps served as guides to the water quality sampling team who chose the following sampling locations:

- The Piney Creek inlet of the James River arm for background
- The Aunts Creek inlet of the James River arm just downstream of the Branson West WWTP
- The waters surrounding Indian Point for septic system influence
- A location on the Kings River arm near where the river officially becomes designated as part of the lake

The final decisions made by the sampling team were made using the suitability maps, personal lake experience, and logistical considerations, such as boat access points. These "human" and logistical factors led to the only difference between the actual sampling sites and those identified through the suitability analysis: the septic tank effluent sampling near Indian Point rather than Shell Knob.

2.5 Summary and Recommendations for Future Work

Sampling site selection is ultimately determined by human knowledge and experience. GIS information and multicriteria analysis can aid these selections, but should not be used blindly without the human element. Suitability analysis is a valuable tool to systematically provide information for multicriteria decision making. When combined with "local" or onsite information, it guides the final determination of sampling sites, saving substantial time and effort traversing the lake for suitable locations.

The Table Rock Lake project provided unique challenges. The goal was to identify sampling locations that were impacted by a single source type. The geology and topography of the area created a reservoir that was narrow and shallow with shorelines characterized by thin soils. Automated site selection through GIS-based multicriteria analysis needed to account for these factors, even when data directly representing these features were not available. Suitability analysis is data-limited. Lake characteristics or pollutant properties cannot be accurately represented in the GIS process unless representative data are input into the process. In this study, surrogate data had to be used in determining septic tank locations, downstream distances, and lake width. The development of a GIS-multicriteria tool allows the site selection process to include feedback and adjust to new information as it becomes available in improving the site selection. Analyzed samples can provide data on whether samples collected were actually influenced primarily by a single source type or if the location on the lake is impacted by other sources. This information can be assimilated into the suitability analysis to provide revised sampling sites for future sampling campaigns.

3 EVALUATION OF CHEMICAL INDICATORS FOR SOURCE APPORTIONMENT OF PHOSPHORUS

3.1 Introduction

Measurements of chemical and biological indicators can complement land use-based water quality modeling in determining significant sources of phosphorus to lakes. Measurement of phosphorus concentrations in a receiving body of water yields information on the total burden of phosphorus from all sources impacting a receptor site, but it does not identify the source. The measurement of multiple chemical and biological species present in phosphorus-containing sources may provide the additional information needed for source tracking and apportionment.

3.1.1 Indicators

The use of *E. coli* measurements at Higgins Lake to identify failing septic systems as the source of phosphorus is an example of the use of a biological indicator for source tracking of phosphorus inputs (Minnerick 2001).

A chemical and biological mass balance approach to source apportionment methods is based on specific sources of pollution having unique ratios of certain chemical and biological species (such as source signatures). Samples are then collected at locations in the receiving water and analyzed for a suite of species. The concentration of each species at the receptor location is the linear combination of contributions from multiple sources. Equation 3-1 expresses the approach in terms of linear algebra.

$$c_i = \sum_{j=1}^{m} f_{ij} a_{ij} s_j \qquad (3\text{-}1)$$

The concentrations of species i (c_i) at a location in the surface water from multiple sources are determined by:

- The fraction of the total water flux coming from each source (s_j)
- The concentrations of species i in source water j (a_{ij})
- Modification factors (f_{ij}) of the concentrations of the species from each source

Modification factors may be due to retardation or degradation of the species during transport. The mass balance approach can be conducted for multiple sources and multiple species. Equation 1 is written for a total of m sources and n species.

For effective application of the mass balance approach, the source signatures (or source profiles) of different source types must be known or be determined. In Equation 3-1, the source signatures are expressed as a_{ij}. Source signatures can be determined by directly sampling the source, or they can be estimated in an inverse approach from a dataset collected at a receptor location. This inverse approach, known as receptor modeling, uses multivariate statistical analysis methods to estimate the source signatures of the primary factors (or components) contributing to the chemical composition of water at a particular location in the receiving water body.

Once source signatures are established and modification factors are known, phosphorus source apportionment can be conducted. First, the relative contributions of water to a particular location are determined.

The $m \times 1$ vector of relative water fluxes (S) at a given location in the lake can potentially be determined by matrix inversion using the measured $n \times 1$ vector of concentrations at the sampling location (C) and the $n \times m$ source signature (A) and transport modification (F) matrices (Seinfeld 1986).

Phosphorus loadings from each source can then be calculated as the product of the water flux from a source, the concentration of phosphorus in the source, and the modification factor for phosphorus transport from the source.

The success of any source apportionment approach depends on the selection of an appropriate set of identifying indicator species. The term species is used here to refer to both chemical and biological constituents. Ideally, each possible source has a unique identifier species that is only present in that source, but it is more likely that a combination of species will be needed as indicators. Appropriate indicators must meet the requirements in Table 3-1.

Table 3-1
Requirements of Useful Indicators

1	The target species must be detectable using available methods, and the species must be present at concentrations above the method detection limit.
2	The ratios of species to one another must be unique to a given source. Ideally some indicators will be present in certain sources and entirely absent in others.
3	The ratios of species to one another (especially to phosphorus) for a given source must be constant and reproducible.
4	Species must be conservatively transported from the source to the receiving body, or the retardation of transport must be well known. Because phosphate can be retarded in porous media, it will be useful to identify indicators with retardation mechanisms similar to those of phosphate.
5	The rates of degradation of the indicators in the receiving water body must be known or measurable.

In this project, the utility of the selected species was evaluated with respect to the first three requirements:

- Presence of potential indicators in the receiving waters at detectable concentrations
- Uniqueness of source signatures
- Consistent concentration ratios of potential indicators to phosphorus

The potential indicators were not evaluated with respect to the transport and degradation requirements. These two requirements are important and can be evaluated in subsequent work focused on those species that meet the first three requirements.

While the requirements of potential indicators are strict, the number of potential indicators is high. Possible chemical and biological indicators that may be used to identify sources of phosphorus pollution include:

- Metals and other inorganic species
- Natural and synthetic organic compounds
- Microorganisms

The use of different types of indicators offers a complementary approach to identifying sources. Some types of indicators could be more useful than others at distinguishing particular sources. Biological indicators are anticipated to be the most effective at distinguishing among effluents from different types of wastewater treatment systems (for example, central wastewater treatment plants or septic systems). Trace metals are anticipated to be the most useful at distinguishing among various agricultural sources (for example, inorganic phosphate fertilizers or animal waste fertilizers).

Methods for source apportionment are relatively mature in the field of air quality management, and the application of source apportionment methods for water quality management is growing. The following sections describe potential indicators used in the project and review some examples of the use of indicators for pollutant tracking or apportionment.

3.1.2 Trace Metals and Metalloids

Trace metals and metalloids present in phosphorus-rich point and nonpoint pollution sources introduced into the lake can be suitable indicators. Advances in elemental analysis techniques, particularly inductively coupled plasma mass spectrometry (ICP-MS), make it possible to rapidly determine the aqueous concentrations of nearly every metal and metalloid in the periodic table at detection limits of parts per billion or better. While most previous studies of metals and metalloids have focused on toxic elements present at elevated concentrations, elemental analysis for source apportionment can make use of non-toxic elements and/or elements present at low levels. Unlike many organic chemicals and biological species, metals and metalloids cannot be transformed into other species, although they may be subject to biogeochemical cycling within an environmental system. Metals and metalloids are present as ionic species in aquatic systems,

and their transport can be influenced by many of the same reactions that affect the transport of phosphate in aquatic systems. The similarity to phosphate transport behavior is particularly true for anionic species (for example, AsO_4^{3-} and SeO_4^{2-}). Because all of the trace elements have natural as well as anthropogenic sources, it is imperative that contributions of these elements from natural sources be assessed.

Inorganic phosphate fertilizers and animal wastes applied to fields as fertilizer can contain unique signatures of various trace elements. Phosphate deposits that are mined for use in mineral fertilizers can have distinct metal compositions resulting from the geochemical conditions of their formation. Chemical analyses of the composition of inorganic phosphate fertilizers have found significant concentrations of heavy metals, radionuclides, and rare earth elements. Elemental compositions of phosphate fertilizers and manure are significantly different (Hamamo *et al.* 1995; Hu *et al.* 1998; McBride and Spiers 2001). These differences can be used to distinguish among potential sources to a lake. Distinguishing among different types of phosphorus-rich fertilizer sources is important because the export of phosphorus from soils can vary greatly with the type of fertilizer used (Kleinman *et al.* 2002).

Inorganic fertilizer sources of pollution to the Everglades were recently identified using uranium isotope ratios (Zielinski *et al.* 2000). Metals are also present in some agricultural chemicals and in supplements fed to animals in AFOs (Weng *et al.* 2002). For example, aromatic organoarsenic compounds are used as feed additives in poultry production, and their breakdown in the environment may release arsenic as arsenate (As(V)), which is chemically similar to phosphate (Jackson and Bertsch 2001). In some cases, hydrolyzing metal salts, such as alum, are added to animal wastes to control phosphate mobility (Peak *et al.* 2002), and trace elements present in the alum or other salt may provide a unique trace element profile for runoff influenced by animal wastes.

Influxes to surface waters from centralized and decentralized treatment plants can also have unique trace element profiles. Heavy metals commonly found in wastewater treatment plant waters include (Metcalf & Eddy 2003):

- Lead
- Copper
- Selenium
- Cadmium
- Chromium
- Arsenic

Trace elements may be contributed by commercial or industrial activities that discharge to the wastewater treatment system. For example, lead isotopes were used to identify a wastewater outfall as the source of lead pollution in a coastal environment (Kersten *et al.* 1997).

Trace elements may also be added to the water as corrosion products from pipes in the water supply system or as part of water and wastewater treatment processes. A recent study found that the use of alum in water treatment contributes trace amounts of arsenic, cadmium, chromium, copper, manganese, nickel, lead, and zinc. Although the levels at which these elements were present would not pose a health hazard, these elements may be useful for identifying surface waters influenced by discharges from a wastewater treatment plant (Eyring *et al.* 2002).

Trace and major element profiles of the source waters used for public supply, which are ultimately discharged as wastewater, can also provide distinguishing information based upon the type—groundwater or surface water—and specific location of the source.

3.1.3 Prescription and Non-Prescription Drugs

Significant advances in analytical methods have enabled the determination of a wide variety of natural and synthetic organic chemicals in natural waters. These chemicals have received considerable attention with respect to potential health and ecological effects (Erickson 2002; Kolpin *et al.* 2002; Renner 2002), but they may also have value as indicators for source apportionment. Chemicals of interest include:

- Agricultural herbicides and pesticides
- Natural and synthetic hormones
- Antibiotics given to humans and livestock
- Prescription and non-prescription drugs
- Specialty chemicals, such as fire retardants and the surfactants in cleaning products
- Metabolites of these chemicals

A recently completed national study by the USGS tested streams for a suite of 95 organic chemicals. The USGS detected 82 of the chemicals. Most of the streams studied contained multiple compounds. The most frequently detected compounds included (Kolpin *et al.* 2002):

- Cholesterol
- 4-nonylphenol (detergent metabolite)
- Triclosan (antimicrobial disinfectant)
- Caffeine

The analysis of many synthetic organic chemicals at the concentrations found in natural waters requires the use of sophisticated and expensive gas chromatograph-mass spectrometer (GC-MS) and high-performance liquid chromatograph-mass spectrometer (HPLC-MS) instrumentation. Of the prescription and non-prescription drugs investigated in the USGS study, acetaminophen, caffeine, and ibuprofen were among the most frequently observed.

Unlike trace metals and metalloids, organic compounds may be metabolized or degraded during transport and within the lake, which can complicate their interpretation as indicators. For example, Triclosan, which is a common antimicrobial product in many consumer products, and fluorescent whitening agents, which are additives in most laundry detergents, are often considered to be indicators of human waste input (Kolpin *et al.* 2002; Poiger *et al.* 1996). However, both of these compounds are subject to relatively rapid photodegradation (Poiger *et al.* 1996; Singer *et al.* 2002), which may limit the distance over which their transport can be observed. Triclosan, however, is methylated during biological wastewater treatment (Lindstrom

et al. 2002), so methyl triclosan, which is not photochemically oxidized, may be useful as an indicator of input from municipal wastewater treatment facilities.

3.2 Methods and Materials

This section describes where and how samples were gathered and preserved for this study and what the samples revealed.

3.2.1 Sampling Sites in the Table Rock Lake Watershed

Table 3-1 shows the location of Table Rock Lake.

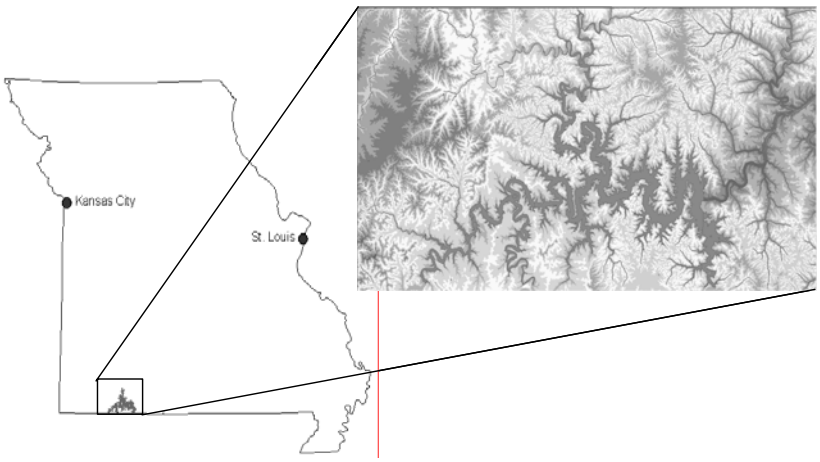

Figure 3-1
Table Rock Lake in Southwest Missouri

Fifteen sampling sites, shown in Figure 3-2 and Figure 3-3, were selected based on:

- Discussions with David Casaletto of Table Rock Lake Water Quality, Inc. (TRLWQ)
- Information gathered during a December 2003 visit by the project team
- Data analysis using GIS, as described in Chapter 2

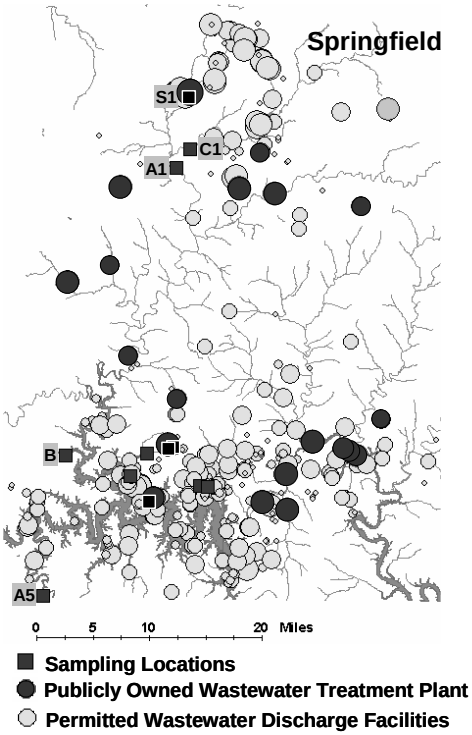

Figure 3-2
Sampling Sites (S1, C1, A1, A5, and B) in the City of Springfield, MO

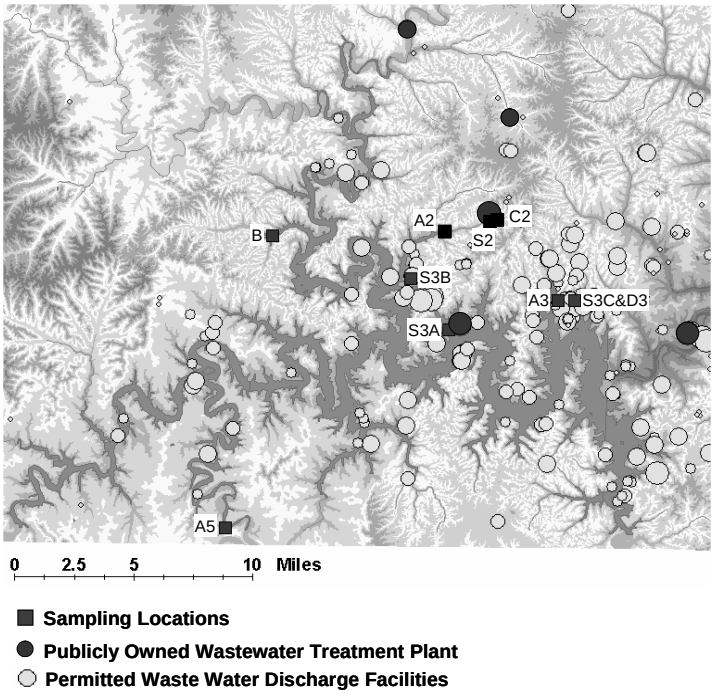

Figure 3-3
Sampling Sites (S2, C2, A2, S3A, S3B, S3C, A3, D3, A5, and B) in Table Rock Lake Watershed, MO

The fifteen sites are classified into five main types as described in Table 3-2.

Table 3-2
Sampling Sites for the Studies Described in Chapters 3 and 4

Site Type		Sites
Sources of wastewater (5)	S1	Effluent of the Springfield Southwest wastewater treatment plant
	S2	Effluent of the Branson West wastewater treatment plant
	S3A	Single compartment septic tank on Joe Bald
	S3B	Single compartment septic tank near Aunts Creek
	S3C	Holding tank in a step system fed by several individual septic systems on Indian Point
Surface water sites likely to be impacted predominantly by a single type of source (4)	A1	Location on the James River site downstream of the discharge point of S1
	A2	Lake site near the flow of Aunts Creek (receiving water of S2) into Table Rock Lake
	A3	Lake site near Indian Point that is close to S3C and in a region with a high density of septic systems
	A5	Site on the Kings River that is likely to be impacted by runoff from animal feeding operations
Control sites upstream of discharges of treated wastewater (2)	C1	Location on the James River upstream of the discharge point of S1
	C2	Site on South Aunts Creek that is upstream of the discharge point of S2
Drinking water supplies that are the influent to wastewater treatment systems (3)	D1	Tap water of the city of Springfield
	D2	Tap water of the city of Branson West
	D3	Well water that supplies a small resort that discharges to septic system S3C
Background site not expected to be impacted by anthropogenic sources (1)	B	Relatively less developed lake site on Piney Creek that is surrounded primarily by a wilderness area

Wastewater source sites are designated as follows:

- The letter S, with S1 and S2 for centralized treatment systems, and S3A–C for three residential septic systems.
- Sites on the lake are designated by the letter A, and the associated number (A1, A2, A3, and A5) corresponds to the number of the source that was anticipated to most significantly influence the site. Site A5 was expected to be influenced by runoff from animal feeding operations and does not have a corresponding source sample. The designation A4 was reserved for a site dominated by inorganic fertilizer runoff, but such a site was not identified in the lake.
- The C designation stands for upstream control sites for the two wastewater treatment plant effluents.
- The D designation stands for drinking water sources used by the water supplies discharging to the wastewater treatment systems.
- Site B is a background site that was not expected to be influenced by any anthropogenic sources.

The average daily treatment volume of Springfield Southwest WWTP is approximately 35 million gallons per day, which discharges into a tributary of the James River and then into Table Rock Lake. This plant is the largest single point source of phosphorus due to its large daily discharge volume. It consists of an old plant and a new plant. The old plant does not have phosphorus removal, and aluminum sulfate is added to the effluent to remove phosphorus by forming precipitates with the phosphorus. The new plant has a biological phosphorus removal process. The effluent of these two plants is disinfected by ozonation before discharge into the James River. The effluent of the Branson West WWTP discharges into South Aunts Creek and then into Table Rock Lake. All three septic tanks are watertight concrete tanks with tar type sealer on the joints.

3.2.2 Sample Collection and Preservation

Samples were collected using a Masterflex® E/STM portable sampler (Cole-Parmer) or a six-foot long grab sampler with a 1 L acid-washed HDPE container attached to the end of it. Teflon® or Tygon® tubing was used with the portable sampler to pump water from the following sites:

- S1
- S2
- S3A
- S3B
- S3C
- A2
- A3
- A5
- B

Separate tubing systems were used for the source sites and the other sites. To avoid contamination between each site, the tubing system was first flushed with deionized water and then with sample water. The grab sampler was used for sampling A1, C1, and C2. A separate HDPE container for the grab sampler was used for each site. Three drinking water supplies (D1, D2, and D3) were directly sampled from faucets into 1 L acid-washed HDPE containers.

Wastewater treatment plant effluents (S1 and S2) were sampled at their discharge points to the environment. Septic tanks were sampled at the points nearest their discharge locations; the tanks for S3A and S3B were sampled at the pump chamber and S3C was sampled from a port that then flowed to the drainfield. Samples for A2, A3, B, and A5 were collected from a boat using a 25-foot Teflon tubing system connected to a portable sampler. At each site, samples were collected from a depth of 3 to 6 feet. Additional water samples were collected for sites B and A3 from deeper regions (15 to 20 feet) in April, July, and October 2004 and for A5 in July 2004.

Samples for each analytical technique were collected in triplicate, except for samples for synthetic organic compounds (SOCs) analysis, which were collected in duplicate. Field blanks for each analytical technique were collected onsite by processing ultra-pure deionized water (18.2 MΩ-cm) following the exact same procedures used for the source and lake water samples. Specific sample collection and preservation procedures for each technique are described in the following sections.

3.2.3 Raw Water Samples

Table 3-3 describes the raw water samples collected.

Table 3-3
Raw Water Samples

Sample	Procedure
Total Phosphorus	A 200 mL portion of unfiltered water was collected in a 250 mL acid-washed glass bottle
Total Trace Elements	A 100 mL portion of unfiltered water was added to a 125 mL acid-washed HDPE bottle and acidified to pH < 2 by adding 1 mL of concentrated (68–70%) trace metal grade HNO_3
SOCs	A 1,000 mL portion of unfiltered water was added into a 1 L HDPE bottle, wrapped with aluminum foil, and then 5 mL formaldehyde was added to prevent biodegradation of SOCs

3.2.4 Filtered Water Samples

Water was filtered using 0.45 μm membrane filters for analysis of dissolved reactive phosphorus, major anions, major elements, and dissolved trace elements, as described in Table 3-4.

Table 3-4
Analysis of Dissolved Reactive Phosphorus, Major Anions, Major Elements, and Dissolved Trace Elements

Sample	Procedure
Dissolved reactive phosphorus	A 100 mL portion of the filtered water was collected in a 250 mL acid-washed glass bottle and stored for less than two weeks
Major anions	A 50 mL portion of the filtered water was collected in a 250 mL acid-washed HDPE bottle and stored for less than two weeks
Major elements and dissolved trace elements	A 100 mL portion of the filtered water was collected in a 250 mL acid-washed HDPE bottle and acidified to pH < 2 by 1 mL of concentrated (68–70%) trace metal grade HNO_3

The following methods and materials were used in collecting the samples:

- All samples were labeled in the field.
- The precise site locations were determined by GPS measurement using an eTrex Vista personal navigator (Garmin).
- The pH, temperature, and dissolved oxygen were measured by an Accumet pH/DO/Temperature meter (Fisher Scientific).
- Conductivity was measured with a digital conductivity meter (Fisher Scientific).
- The location, time of sampling, pH, DO, conductivity, and water temperature were recorded onsite.
- Samples collected in the field were transported to Washington University laboratories by the members of the project team who performed the sampling.
- All samples were kept cold in ice chests from the time of sampling to the time of their arrival at Washington University and were then stored in refrigerators until analysis.
- Sampling was conducted once per season (April 2004, July 2004, October 2004, and January 2005) for all sites for a whole year.
- There were four sampling events for the whole project.

Table 3-5 presents the exact sampling dates, reservoir levels during sampling, flow rates of the James River just before it enters the lake, and local precipitation during and preceding the sampling campaigns.

Table 3-5
Hydrologic Conditions During and Preceding Sampling

Sampling Dates	Reservoir Elevation (ft) [a]		Flow Rate at Galena (cfs)		Precipitation (in.) [a]		Reservoir Inflow (DSF) [a]	
	Min	Max	Min	Max	Sampling Week	Preceding Week	Sampling Week	Preceding Week
April 12–16, 2004	914.04	914.24	586	787	0	0.80	11378	15542
July 12–16, 2004	915.37	916.75	266	337	0.22 [c]	1.01	16281	28154
October 11–14, 2004	913.10	913.24	129	321	1.39	1.47	5539	6262
January 17–21, 2005 [b]	918.34	916.45	1970	3630	0	1.56	32457	NA

[a] Reservoir elevation, precipitation in basin, and reservoir inflows are from the Table Rock Lake Monthly Reports prepared by the US Army Corps of Engineers Little Rock District.

[b] The January 2005 Monthly Reservoir Report was not available at the time of this writing. Precipitation, inflow, and reservoir elevation were provided for the dates January 17–21 by Little Rock District Army Corps of Engineers Personnel. The precipitation for the preceding week was determined from other data available at Springfield, Missouri.

[c] During the July sampling campaign, the only precipitation occurred on the last sampling day and no lake sites were sampled during that time.

Table Rock Lake is a reservoir operated by the Army Corps of Engineers. The normal pool for the reservoir is 915 ft. The precipitation measurements are amounts for the Table Rock Lake Basin reported by the US Army Corps of Engineers Little Rock District. The reservoir inflow data reported by the Corps of Engineers are given in units of day-second-foot (DSF); 1 DSF = 86,400 ft^3, which is the volume of water that would accumulate in one day from a flow rate of 1 ft^3/s (cfs). Additional streamflow data in the basin are from the US Geological Survey gauging station at Galena, Missouri (No. 07052500), which is the station on the James River closest to Table Rock Lake.

In addition to covering different seasons, the sampling campaigns included a range of hydrologic conditions. The April sampling campaign was conducted when the lake was just below normal pool and the inflows were affected by moderate precipitation in the preceding week. The July sampling campaign was conducted when the reservoir level was high and the preceding week had included high inflows, which were largely the result of releases from Beaver Reservoir upstream and not from recent precipitation. The October sampling campaign was conducted during a week of heavy precipitation and was also preceded by high precipitation; however, the lake inflows in the preceding week were low and the lake level was also low. The January sampling campaign had the highest streamflows, reservoir inflows, and lake levels of the four sampling campaigns.

3.2.5 Sample Analysis

The dissolved concentrations of seven major anions (F^-, Cl^-, Br^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, NO_2^-) were determined by ion chromatography (Dionex DX-600) by Standard Method 4110 (Eaton *et al.* 1998). Dissolved concentrations of four selected major elements (Na, K, Mg, and Ca) were determined by inductively coupled plasma optical emissions spectroscopy (Varian Liberty II) according to Standard Method 3120 (Eaton *et al.* 1998). Dissolved and total trace elements were determined by inductively coupled plasma mass spectrometry (Finnigan Element 1, Thermo Electron Corporation) according to Standard Method 3125 (Eaton *et al.* 1998).

Dissolved reactive phosphorus (DRP) concentrations were determined colorimetrically using the ascorbic acid method (Lambda 2S spectrometer, Perkin Elmer) according to Standard Method 4500-P (Eaton *et al.* 1998). A quartz spectroscopy cell with a 10 cm light path was used and the method detection limit was 1 ppb. For measurement of total phosphorus (TP), water samples were first digested by the persulfate digestion method according to Standard Method 4500-P (Eaton *et al.* 1998). The digested samples were then analyzed by the ascorbic acid method.

Four synthetic organic compounds (caffeine, acetaminophen, trimethoprim, and sulfamethoxazole) were analyzed by a modified HPLC method (Cahill *et al.* 2004). These compounds are excreted following human consumption of beverages and over-the-counter and prescription drugs. The compounds have been found in domestic wastewater and ambient surface waters.

Water samples were first concentrated by a factor of 500 by solid phase extraction (SPE). A 1,000 mL sample was passed through a 500 mg SPE cartridge (Oasis HLB cartridge, Waters) by vacuum to extract SOCs onto the cartridge. Ten mL methanol was eluted through the cartridge to wash off the extracted SOCs. The collected methanol containing the extracted SOCs was further concentrated to about 0.2 mL by nitrogen sparging, and its volume was brought back to 2 mL by adding ultra-pure water. The concentrated sample was then ready for injection into HPLC for UV detection. The chromatographic conditions were:

1. Column: Metasil Basic 3 μm, 150 mm × 2 mm, reverse-phase octylsilane (C8) column

2. Mobile phase: channel A—80% acetonitrile: 20% water channel B—pH 3.3 buffer, made by adding 3 mL formic acid to 1 L water and adjusting to pH 3.3 by adding ammonium formate

3. Gradient elution: flow rate 0.25 mL/min, 10% solution A linearly changed to 90% solution A from 0 min to 15 min

4. Detection wavelength of UV lamp: 255 nm

5. Injection volume: 100 μL

6. Temperature: ambient temperature, about 20 °C (68 °F)

The major problem with this HPLC method is the interference from numerous dissolved organic compounds (DOCs) present in the sample, many of which are also concentrated by SPE and detected by the UV detector. Figure 3-4 shows a chromatogram for the analysis of a sample collected at site S3C in July 2004.

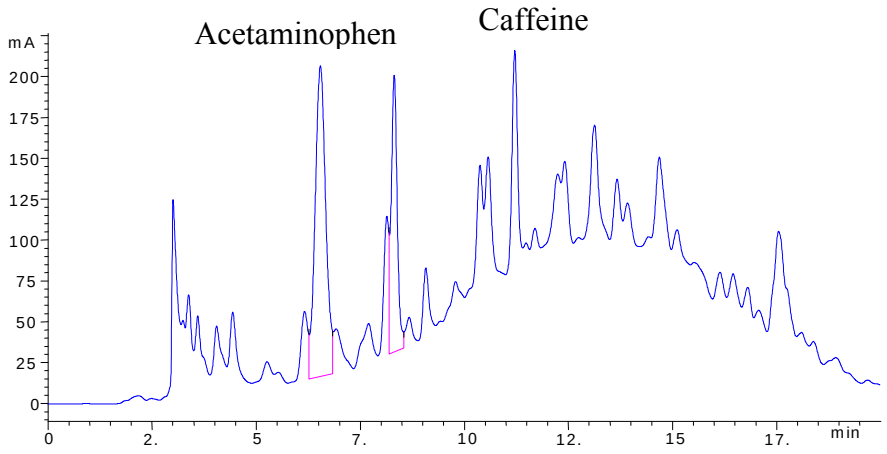

Figure 3-4
Chromatogram of an S3C Sample

There are many dissolved organic compounds shown in this chromatogram. The retention times of some compounds are close to those of the target compounds. By checking the UV spectrum of each peak, only the identity of acetaminophen and caffeine can be confirmed. By comparing this chromatogram with the chromatogram of standards, the baseline drift was considerably higher, which was possibly caused by the matrix effect of water samples after SPE concentration. Matrix effects will reduce method detection limits and may make the detection of these low-concentration organic compounds difficult or impossible.

In summary, the major disadvantages of UV detection are:

- Cannot provide qualitative information of target compounds
- Subject to substantial interferences

Caffeine was detected (from 4.8 to 136 µg/L) in several samples from septic tanks. Acetaminophen (132 µg/L) was detected in one septic system using this HPLC method. No SOCs were detected at the rest of the sites. To measure low concentrations of SOCs present in environmental water samples, mass spectrometric detection is required. Twelve SOC samples were sent from the January 2005 sampling campaign to Dr. Steven Zaugg of the USGS National Water Quality Laboratory in Denver, Colorado for GC-MS analysis of a set of wastewater compounds using a method developed by Dr. Zaugg and colleagues (Brown *et al.* 1999). But the laboratory misplaced the samples and could not analyze them.

3.2.6 Quality Assurance

The project was conducted strictly according to the Quality Assurance Project Plan submitted and approved in March 2004. All samples for the same site in one sampling event were collected in triplicate or duplicate as described previously. Field blanks were collected to control the quality of sampling and analysis. Chemical analysis was performed according to the standard operating procedures for each instrument. An internal standard of 1 ppb indium was used for ICP-MS analysis of trace elements.

3.2.7 Data Analysis

Successful source apportionment of phosphorus requires finding a set of suitable indicator species (Table 3-1). Suitable indicators may be either a single unique species or a unique combination of several species that are characteristics of a certain source (such as a source signature). As discussed in the Introduction, there are five requirements for a useful indicator. In this project, the potential indicators were evaluated with respect to the following three requirements:

- Presence of potential indicators in the receiving waters at detectable concentrations
- Uniqueness of source signatures
- Consistent ratios of potential indicators to the phosphorus concentrations

The potential indicators were not evaluated with respect to the transport and degradation requirements. These two requirements are important and can be evaluated in subsequent work focused on those species that meet the first three requirements.

Principal component analysis (PCA) was used to find source signatures (a unique combination of several species that are characteristics of a certain source). PCA is a multivariate statistical method that examines the relationships within a large set of variables (Henry *et al.* 1984; Jackson 1991). PCA is widely used in interpreting environmental data (Phillips *et al.* 1997; Veltkamp *et al.* 1996; Zitko 1989; Zitko 1994). PCA transforms many interrelated variables into a smaller set of independent components, or factors, that account for the variance in the data set. The variables within the same factor are usually highly correlated.

Factors are extracted from a correlation matrix based on the average and standard deviation of each z-scored variable (that is, chemical species). PCA provides as many factors as there are variables, and only a small number of factors are retained for further rotation after examination of latent root and scree plot. The purpose of the rotation is to redistribute a particular variable's presence from several factors into one to further emphasize relationships among the data. Varimax rotation is often used because it preserves the orthogonality between the factors, which generally simplifies data interpretation.

A factor loading describes the degree to which a variable contributes to a particular factor. A factor score describes the degree to which a sample reflects high concentrations of the group of variables contained in a factor. Based on factor loadings and factor scores, each factor can be attributed to certain source(s).

PCA was performed using Systat 10 software (Systat Software, CA, USA) on the water quality matrix obtained through four sampling events. There were a total of 67 sites (cases) sampled and analyzed during the project (four sampling seasons and about 17 sites per season). The measurements of all chemical species for each site are shown in Appendix A. Not all measured chemical species were included in the water quality matrix for PCA. Since the concentrations of total trace elements and dissolved trace elements were very close, only total trace elements were used in PCA. Similarly, since dissolved reactive phosphorus and total phosphorus followed the same patterns, only TP concentrations were used in PCA. Chemical species with little variability among sites were also excluded. The following nineteen parameters were included in PCA:

- Total Phosphorus
- Sr
- Ba
- V
- Cr
- Co
- Ni
- Cu
- Mo
- As
- Ca
- Mg
- Na
- F^-
- Cl^-
- Br^-
- SO_4^{2-}
- PO_4^{3-}
- NO_3^-

While the pre-selection of chemical species could have some effect on PCA, it was carried out carefully to avoid biasing the results. To isolate interpretable factors, PCA was performed on:

- All sites
- Just on source sites
- On all sites except the source sites

Performing the PCA on all sites except for the sources avoided biasing the results based on the high concentrations present in the sources. Only species above detection limits were included in the PCA.

3.3 Results

The following sections describe the chemical and PCA results of the samples.

3.3.1 Chemical Measurements of All Sampling Sites

Water quality for all sites is summarized in Appendix A. Only the average concentration of triplicate or duplicate measurements is shown. The standard deviations are not shown because

they were usually relatively small (less than 5%). The spatial and temporal distributions among all sites for each parameter are discussed in the following section.

3.3.1.1 pH, DO, Temperature, and Conductivity

The difference in pH among sites was small:

- Most pH values were within pH 7.0–8.0.
- Generally, river sites (A1, C1, and A5) and lake sites (C2, A3, and B) had higher pH values than source sites (S1, S2, S3A, S3B, and S3C).
- Seasonal variation of pH for the same site was not significant.

DO varied greatly among sites:

- S1 had the highest DO values (greater than 20.0 ppm) of all sites because the effluent was disinfected with ozone before its discharge.
- The DO values of the three river sites and three lake sites were the next highest, and were generally almost to the saturation concentration (that is, in equilibrium with atmospheric oxygen).
- The DO values of the three residential septic tanks (S3A, S3B, and S3C) were the lowest because of the high BOD loadings of the tanks and anaerobic conditions desirable for septic tanks.
- The DO values of the three drinking water supplies (D1, D2, and D3) varied randomly and were generally higher than those of septic tanks and lower than those of other sites.
- The DO values of lake sites and river sites were highest in January and lowest in July because of the water temperature difference between different seasons; saturated DO increases with decreasing temperature.

Water temperature varied with the seasons:

- Water temperature was measured after the water was pumped from the lake and the water temperature increased while flowing through the tubing; thus, these lake sites had very high temperatures in July 2004.
- The source sites and drinking water supplies had less temperature variation than the river and lake sites; however, the difference among river and lake sites for the same sampling event was not large.

Conductivity varied significantly among sites:

- The five source sites had high conductivity values (greater than 1,000 µS/cm), corresponding to high total dissolved solids (TDS).
- S3B and S3C had the highest conductivities of all sites.

- All other sites had lower conductivity (300–500 μS/cm), except for D3, the well water on Indian Point, which had high conductivity values close to those of source sites.
- Seasonal variation in conductivity for each site was not great.

3.3.1.2 Total Phosphorus and Dissolved Reactive Phosphorus

The seasonal variations of total phosphorus concentrations were large for S2, C2, S3A, and S3B. Total phosphorus concentrations at the rest of the sites did not vary significantly over the year, except in January 2005. During that time, the three river sites (A1, C1, and A5) had much lower concentrations, and site B had a higher concentration in January 2005 than during other seasons. In January 2005, Table Rock Lake had received heavy water influxes, including flows from Beaver Reservoir upstream. If Beaver Reservoir has higher phosphorus concentrations than Table Rock Lake, then the influx could affect sites in Table Rock Lake closest to the main body of the lake. However, if the total phosphorus was the result of upstream reservoir inflows, higher total phosphorus at sites A2 and A3 would be expected.

Total phosphorus varied significantly among sites. The sites can be classified into five groups according to their total phosphorus concentrations as shown in Table 3-6.

Table 3-6
Distribution Pattern of Total Phosphorus Concentration Among Sites

Total Phosphorus Concentration	Sites
1.2 ppm–11.2 ppm	3 septic tanks: S3A, S3B, and S3C
60 ppb–1.24 ppm	2 WWTP effluents: S1 and S2
17 ppb–95 ppb	3 river sites: A1, C1, and A5
7 ppb–82 ppb	C2, A2 and B
<10 ppb	A3, D1, D2, and D3

The three septic tanks had the highest concentrations. The wastewater treatment plant effluents (S1 and S2) had the second highest concentrations. Total phosphorus concentrations of S1 were consistently around 100 ppb, indicating phosphorus removal operations at the plant were functioning properly. Total phosphorus concentrations of S2 varied more and were higher than those of S1. Total phosphorus concentrations of the three river sites (A1, C1, and A5) were the third highest. A3 and the three drinking water supplies had the lowest concentration of total phosphorus (below 10 ppb).

By comparing the total phosphorus concentrations of four related sites—S1, C1, A1, and D1—the trend of S1>A1>C1>D1 on the upper James River indicates that the WWTP effluent is a significant source of phosphorus pollution. Source S1 had higher total phosphorus and its discharge volume was significant relative to the flow rate of the James River. Thus total phosphorus concentrations of downstream site A1 exhibit the influence of S1.

In contrast, S2 did not have an observable effect on the downstream site A2 in comparing the four related sites—S2, A2, C2, and D2. A2 is a lake site connected to the main water body of Table Rock Lake, where the total phosphorus concentrations were generally low (around several ppb). S2 has a much smaller discharge volume than S1 and its phosphorus input can be diluted by water from the rest of the lake.

A similar situation is observed for the three septic systems. Although the three septic systems had high concentrations of total phosphorus, the phosphorus either did not reach the lake because it was captured in the drainfield, or the concentrations were low due to dilution with the rest of the lake.

Dissolved reactive phosphorus concentrations were generally lower than total phosphorus concentrations for the same site and same reason. The distribution pattern of dissolved reactive phosphorus concentrations was very similar to that of total phosphorus concentrations.

3.3.1.3 Total and Dissolved Trace Elements

The differences between total and dissolved trace elements concentrations were not significant for most sites. The 15 trace elements can be classified into 4 groups according to their concentrations in source samples. (Table 3-7). The distribution pattern of each trace element among sites is discussed later. All of the trace elements have both natural and anthropogenic sources and none are completely unique to a given source.

Table 3-7
Classification of 15 Trace Elements According to Their Concentrations in Sources

Trace Elements	Concentration Rank
Sr, Ba, and Zn	Highest
Mo, Cu, and As	Second highest
Ni, Co, and V	Third highest
Sb, Pb, U, Cr, and Hg	Lowest

Strontium (Sr)

S3A had the highest Sr. The Sr concentrations varied considerably compared to other sites. Sr concentrations at S1, A1, and C1 were the second highest. Sr concentrations at the rest of the sites were very close. The difference in Sr concentration between sources and receiving surface water bodies was not significant, indicating that Sr concentrations are mainly controlled by natural geological sources. For example, the Sr concentrations at the related sites S1, A1, C1, and D1 were very similar. Sr concentrations at S2, A2, C2, and D2 were similar. And Sr concentrations at A3 and D3 were similar. Sr concentrations of drinking water supplies were the lowest. Sr concentrations of sources were the highest among the related site groups, indicating that human activities still had some Sr input.

Barium (Ba)

S1, S2, D1, D2, and D3 had lower concentrations of Ba than corresponding river or lake sites, indicating Ba can be removed during the water and wastewater treatment processes. In a region rich in carbonate minerals, barium will be naturally present in groundwater and surface water. For example, Ba^{2+} can be removed by SO_4^{2-} from water by precipitation of insoluble $BaSO_4$. Ba concentrations of the three septic systems were not higher than at the three lake sites. However, Ba concentrations were not as low as those of S1 and S2 because they have lower SO_4^{2-} concentrations, possibly due to the anaerobic conditions of the septic systems.

Zinc (Zn)

Seasonal variation of Zn was large. Generally, source sites had higher Zn concentrations than other sites. Zinc can have both natural and anthropogenic sources, although zinc's use in metallic materials can explain the higher concentrations in the source samples. The three lake sites had the lowest Zn concentrations of all sites.

Molybdenum (Mo)

S1 had the highest Mo concentrations among all sites. The Mo concentrations of the other four source sites were not significantly different than the rest of the sites. The three lake sites had the lowest Mo concentrations. Most sites had the highest Mo concentrations during the October 2004 sampling campaign. Molybdenum is a naturally occurring element, but it is also used as an alloy in several compositions of steel.

Copper (Cu)

The three drinking water supplies had the highest Cu concentrations. The main source of Cu is probably the pipe in the water supply distribution system. The three septic tanks had the second highest Cu concentrations, probably from copper plumbing in contact with the water used on site. The effluents of two WWTPs had much lower Cu concentrations than drinking water or the effluents of septic tanks, indicating that a large portion of Cu was removed from the water by aerobic wastewater treatment processes. The three lake sites had the lowest Cu concentrations (less than 1 ppb). Cu concentrations of the river sites were also very low and only slightly higher than those of the lake sites. Copper is also present in natural waters, but the high concentrations in the source samples indicate that anthropogenic sources are dominant.

Arsenic (As)

The five source sites had the highest As concentrations. Arsenic concentrations of drinking water supplies were the lowest (below 1 ppb). River sites and lake sites also had very low As concentrations (mostly around 1 ppb). Arsenic can result from both geological and several anthropogenic sources, including food in the human diet. Originally it was hypothesized that the use of arsenic in organoarsenic compounds used in poultry production would make arsenic a

useful indicator for agricultural runoff, but no trends were found that would indicate such a source.

Nickel (Ni), Cobalt (Co), and Vanadium (V)

The elements nickel, cobalt, and vanadium can have both geological and industrial sources. Most sites had very low Ni concentrations (below 1 ppb). However, the five source sites had considerably higher Ni concentrations. Co concentrations followed a similar distribution pattern to that of Ni concentrations, but were usually a little lower. V concentrations were generally low and seasonal variation of V was small for all sites. S1 and S2 had the highest V concentrations of all sites. The drinking water supplies and septic tanks had the lowest concentrations of V (most below 0.5 ppb).

Antimony (Sb), Lead (Pb), Uranium (U), and Chromium (Cr)

Antimony, lead, uranium, and chromium have both geological and anthropogenic sources. Concentrations were very low, and most values were below 1 ppb. Sb concentrations of source sites were not higher than those of other sites. The three drinking water supplies and S1 had higher Pb concentrations than other sites, possibly derived from distribution system pipe. Sometimes, a good response and calibration curve for Pb could not be obtained, which resulted in no detection of Pb. D2 and D3 had higher concentrations of U than the other sites had. The concentrations of Cr were higher in the septic tanks than at the other sites.

Mercury (Hg)

The detection sensitivity and linearity of Hg analysis by ICP-MS is not as good as for other trace metals. Mercury is present in natural geological materials, although anthropogenic sources may be dominant. Important mercury sources are from industrial wastewaters and from atmospheric deposition of mercury emitted from coal combustion and other air emissions sources. The responses of all samples were not significantly different than the lab blanks; thus, Hg was not detectable in all sites.

3.3.1.4 Major Elements: Ca, K, Mg, and Na

All of the major elements have both natural and anthropogenic sources and none are completely unique to a given source.

Calcium (Ca)

Seasonal variation of Ca concentrations for all sites was not significant. S3A had the highest Ca concentrations. Ca concentrations of other sites were very close, generally ranging from 30 to 70 ppm. Natural sources will dominate calcium concentrations, especially in a region with an abundance of calcium carbonate minerals in limestone.

Potassium (K)

D3 had the highest concentrations of K among all sites. S2, S3A, S3B, and S3C had the second highest concentrations of K. ICP-OES was not sensitive for K measurement, and often a good response and calibration curve could not be obtained. Potassium is primarily from natural sources.

Magnesium (Mg)

Seasonal variation of Mg was low. Patterns in Mg concentrations were generally similar to those in Ca concentrations. S3A had the highest Mg concentrations. The three septic tanks, S2, and the three drinking water supplies had higher Mg concentrations than the rest of the sites. Similar to calcium, magnesium inputs to the reservoir are dominated by natural geological sources.

Sodium (Na)

Seasonal variation of Na was low. Sodium has natural sources, but it can also be enriched by anthropogenic processes. The five source sites and D3 had the highest Na concentrations. The rest of the sites had much lower concentrations of Na, ranging from 2 to 10 ppm, except for A1. By comparing Na concentrations of S1, A1, C1, and D1, A1 was observed to have significantly higher Na concentrations than C1 due to the large input from S1. D2 of Apr 2004 and D3 of January 2005 were tap water that had undergone water softening treatment by ion exchange. Thus they had high Na concentrations but very low Ca and Mg concentrations.

3.3.1.5 Major Anions: F^-, Cl^-, SO_4^{2-}, Br^-, NO_3^-, NO_2^-, and PO_4^{3-}

All of the major anions have both natural and anthropogenic sources, and none are completely unique to a given source.

Fluoride ion (F^-)

F^- concentrations at most sites were low, and seasonal variation was small. High concentrations of fluoride were occasionally observed. For example, D1 of April 2004, D2 of January 2005, and S3A of April 2004 and January 2005 had high concentrations. S1 had higher concentrations of F^- for all four seasons than the other sites, probably from fluoride addition at the municipal water treatment plants. The influence of high F^- in S1 is also apparent downstream, with elevated F^- concentrations in A1.

Chloride ion (Cl^-)

Seasonal variation of Cl^- concentrations at most sites was not significant. Chloride has both natural and anthropogenic sources. The five source sites had the highest concentrations of Cl^-. Most values were around 100 to 300 ppm. Among them, S3A had the highest concentrations.

S3B had the lowest. A1 had higher concentrations than C1 due to the input from S1; however, A2 did not have higher concentrations than C2. River sites, lake sites, and drinking water supplies had low concentrations of Cl^-, from 1 to 10 ppm. A1 and D3 had higher Cl^- concentrations.

Sulfate (SO_4^{2-})

Seasonal variation of SO_4^{2-} concentrations was low. Sulfate has both natural and anthropogenic sources. The effluents of the two WWTPs (S1 and S2) had the highest concentrations of SO_4^{2-}. Most values were around 50 to 100 ppm. SO_4^{2-} concentrations at the three septic tanks and at D3 were the second highest. Similarly to Cl^-, A1 was observed to have higher SO_4^{2-} concentrations than C1 due to the input of S1; however, A2 was not observed to have higher concentrations than C2.

Bromide ion (Br^-)

High concentrations of Br^- were present in S1 all year, ranging from 24.1 to 38.6 ppm. Since C1 and D1 did not have detectable Br^-, relatively high concentrations of Br^- present in A1 were derived exclusively from S1. Three lake sites—A2, A3, and B—had consistently low concentrations of Br^- all year (0.2 to 0.8 ppm). Br^- was below the IC detection limit in S2, the three drinking water supplies, C2, and A5. It was only occasionally detected in the three septic tanks at low concentrations. The reason A5 did not have detectable Br^- concentrations was that A5 is near to where Kings River flows into Table Rock Lake, which is relatively far from the entrance point of the James River into Table Rock Lake, compared to the other three lake sites (A2, A3, and B). Thus S1 might be the main Br^- source for all lake sites. The source of Br^- in S1 is not clear.

Nitrate (NO_3^-) and Nitrite (NO_2^-)

NO_3^- concentrations varied randomly between seasons for most sites and were typically highest in April 2004 and January 2005. For example, S1 had the highest concentrations of NO_3^- in April 2004 (48 ppm) and January 2005 (27 ppm) among all sites. Nitrate concentrations of S1 are not regulated. The undeveloped lake site (B) had no detectable NO_3^- in July 2004 and October 2005, while it had relatively high concentrations in April 2004 and January 2005. D3, A1, C1, C2, and S3A had consistently high NO_3^- concentrations. A2, D2, and A3 had the lowest NO_3^- concentrations. NO_3^- concentrations at S2, S3B, and S3C were generally low. NO_2^- was not detected in most samples because NO_2^- is usually oxidized to NO_3^- in aquatic systems.

Phosphate (PO_4^{3-})

PO_4^{3-} concentrations correlated with total phosphorus concentrations. Septic tanks had the highest concentrations of PO_4^{3-}. Most lake and river sites had low concentrations of PO_4^{3-} that were undetectable by current IC methods.

3.3.2 Analysis With PCA

The following sections discuss the results of PCA analysis.

3.3.2.1 PCA Results on All Sites

PCA was performed on 67 cases (four seasons at approximately 17 sites per season) on the following 19 parameters:

- Total phosphorus
- Sr
- Ba
- V
- Cr
- Co
- Ni
- Cu
- Mo
- As
- Ca
- Mg
- Na
- F^-
- Cl^-
- Br^-
- SO_4^{2-}
- NO_3^-
- PO_4^{3-}

Six principal components with eigenvalues greater than 0.9 were extracted and rotated with Varimax rotation. Table 3-8 shows the factor loadings of six extracted factors and sampling sites that most strongly follow the patterns of the factor. Only factor loadings higher than 0.4 are shown in Table 3-8.

The major sites influencing each factor were identified by calculating the factor score of each site on each factor. Table D-1 in Appendix D shows the factor scores of each site on these six factors.

Table 3-8
Factor Loadings of Six Factors for PCA on All Sites

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
TP		0.91				
Sr	0.89					
Mo			0.47		0.66	
Ba	0.62			0.50		
V			0.49	0.54		
Cr		0.46			0.74	
Ni	0.90					
Co	0.90					
Cu				-0.88		
As	0.40				0.46	0.57
Ca	0.83					
Mg	0.63			-0.61		
Na	0.56	0.54				0.42
F^-	0.38				0.70	
Cl^-	0.87					
SO_4^{2-}			0.62			0.67
Br^-			0.88			
NO_3^-			0.84			
PO_4^{3-}		0.89				
Variance expressed	29%	14%	13%	10%	12%	8%
Major Source(s)	S3A, S3C	S3A, S3B, S3C	S1, A1	A1, C1, A2, C2, A3, A5, and B	S3A, S3B, S3C, and S1	S3A, S2

1. S3A had high factor scores and S3C had moderate scores on Factor 1 and they are the dominant sites contributing to Factor 1 with high loadings of Sr, Ni, Co, Ca, Mg, and Cl^-, which means that septic tanks usually had higher concentrations of these chemical species than other sites had.

2. S3A, S3B, and S3C are the major sources contributing to Factor 2, which has high loadings of total phosphorus and PO_4^{3-}. This is consistent with the fact that phosphorus concentrations were higher in septic tanks than at other sites.

3. S1 and A1 are the major sites contributing to Factor 3, which has high loadings of Br^-, NO_3^- and SO_4^{2-}. Factor 3 represents a unique signature for S1, and this signature was reflected in its downstream site A1.

4. Factor 4 has moderate loadings of Ba and V and high negative loadings of Cu and Mg. Its major sources are the river and lake sites (A1, C1, A2, C2, A3, A5, and B). Negative factor loadings for Cu mean low Cu concentrations. This is consistent with measured concentrations of Cu and Mg at these sites that are lower than at the source sites and in drinking water supplies.

5. S3A, S3B, S3C, and S1 are the major sites contributing to Factor 5, which has high loadings of Cr, Mo, F^- and As.

6. S2, S3A, S3B, and S3C are the major sites contributing to Factor 6, which has high loadings of Na, SO_4^{2-}, and As. Factor 5 and Factor 6 can be deemed to represent variations associated with common characteristics among source sites.

3.3.2.2 PCA Results on Source Sites

PCA was performed on 20 source cases (5 sources sampled during four seasons) on the following 18 parameters:

- total phosphorus
- Sr
- Ba
- V
- Cr
- Co
- Ni
- Cu
- Mo
- As
- Ca
- Mg
- Na
- F^-
- Cl^-
- Br^-
- SO_4^{2-}
- NO_3^-

Phosphate (PO_4^{3-}) was not included because it was highly correlated with total phosphorus and did not provide additional information as revealed by PCA on all sites. To obtain statistically meaningful results, PCA has a minimum requirement for the case to parameter ratio and the ratio here is likely too small.

Five principal components with eigenvalues greater than 0.9 were extracted and rotated with Varimax rotation. Table 3-9 shows the factor loadings of five extracted factors and their corresponding major sources. Only factor loadings higher than 0.4 are shown in Table 3-9. The major contributing sites for each factor were identified by calculating the factor score of each site on each factor. Table D-2 in Appendix D shows the factor scores of each site on these five factors.

Table 3-9
Factor Loadings of Five Factors for PCA on Five Source Sites

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
TP		-0.80			
Sr	0.57			0.74	
Mo			0.54		0.60
Ba	0.60			0.65	
V					0.66
Cr			0.85		
Ni	0.53			0.81	
Co	0.55			0.71	
Cu		-0.80			
As			0.80		
Ca	0.91				
Mg	0.88				
Na	0.86				
F^-				0.77	
Cl^-	0.92				
SO_4^{2-}		0.79			0.40
Br^-					0.89
NO_3^-					0.81
Variance expressed	25%	15%	11%	19%	15%
Major Source(s)	S3A and S3C	S1 and S2	S3A	S3A	S1

Factor 1 has high loadings of Sr, Ba, Ni, Co, Ca, Mg, Na, and Cl^- and its dominant sources are S3A and S3C. Factor 1 primarily represents the common characteristics of septic tanks. This factor is quite similar to the first factor in Table 3-8.

Factor 2 has high loading of SO_4^{2-} and high negative loadings of TP and Cu. S1 and S2 are the dominant sites contributing to Factor 2, which is consistent with the observations of WWTP effluents having lower concentrations of Cu and total phosphorus and higher SO_4^{2-} than the septic system effluents had.

S3A is the major source contributing both to Factor 3 and Factor 4. Factor 3 has relatively high loadings of Mo, Cr, and As. Factor 4 has relatively high loading of Sr, Ba, Ni, Co, and F^-. This is consistent with S3A having relatively higher concentrations of these chemical species than the other source sites.

Factor 5 has high loadings of Br^- and NO_3^- and moderate loadings of Mo and V. The major contributing site is S1. This factor is almost the same as the third factor in Table 3-8.

3.3.2.3 PCA Results on Lake Sites

When the five source sites are included in PCA, the distribution pattern of these chemical species among river sites, lake sites, and drinking water supplies are potentially masked because the five source sites had much higher concentrations than the rest of the sites. To further explore the relationship between these chemical species, PCA was performed using 47 cases (four seasons and about 12 sites per season) on the lake sites, river sites, and drinking water supplies on the following the 18 parameters:

- total phosphorus
- Sr
- Ba
- V
- Cr
- Co
- Ni
- Cu
- Mo
- As
- Ca
- Mg
- Na
- F^-
- Cl^-
- Br^-
- SO_4^{2-}
- NO_3^-

Table 3-10 shows the factor loadings of six extracted factors and their corresponding major sources. Only factor loadings higher than 0.4 are shown in Table 3-10. The major source of each factor was identified by calculating the factor score of each site on each factor. Table B-1 shows the factor scores of each site on these five factors.

Table 3-10
Factor Loadings of Six Factors for PCA on All Sites Except Source Sites

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
TP						0.78
Sr	0.49				0.74	
Mo	0.88					
Ba					0.81	
V	0.39				0.52	0.45
Cr	0.76					
Ni	0.46	0.47				0.60
Co						0.83
Cu			0.88			
As		0.55				0.55
Ca					0.86	
Mg			0.92			
Na		0.79				
F^-				0.92		
Cl^-		0.78				
SO_4^{2-}		0.80				
Br^-				0.89		
NO_3^-		0.45				0.59
Variance expressed	13%	15%	12%	10%	17%	16%
Major Source(s)	A1, C1, D1, and B	A1 and D3	D2 and D3	A1	A1, C1, D1, and C2	A1, C1, and A5

Factor 1, Factor 2, and Factor 5 are not easily interpretable. Factor 3 has high loadings of Cu and Mg and the major contributing sites are drinking water supplies (D2 and D3). This is consistent with the fact that D2 and D3 had higher concentrations of Cu and Mg among these sites. Factor 4 has loadings of Br^- and F^- and the major contributing site is A1. This is consistent with A1 having elevated values of Br^- and F^- among these sites. Factor 6 has high loadings of TP, Ni, Co, Ca, and NO_3^- and the major contributing sites are three river sites A1, C1, and A5. This is consistent with river sites having higher concentrations of these chemical species than the lake sites and drinking water supplies.

3.4 Discussion

The following sections discuss the enrichment of chemical species in source sites and evaluate indicator ratios.

3.4.1 Enrichment of Chemical Species in Source Sites Compared to Their Corresponding Drinking Water Supplies

Species that may be useful indicators of wastewater influence will be those with concentrations that are affected (either enriched or depleted) by human use. By comparing the concentrations of the investigated chemical species in the drinking water supply and in the corresponding source site, better knowledge can be gained of the enrichment processes of these chemical species.

One way to compare is to normalize all chemical species to a certain chemical species that has similar concentrations in the source site and its corresponding drinking water supply. This chemical species should be conservative during transport from the drinking water supply to the source sampling location and there should be minimal human input during the transport. By comparing the normalized values for a certain species between the corresponding source and drinking water supply, one can know whether the chemical species is enriched or depleted during the transport of drinking water to the source site. An enrichment factor of a species can be defined as the ratio of its normalized concentration in the source to its normalized concentration in the corresponding drinking water supply for the same season.

Ca was found to be relatively consistent among all sites and thus all species were normalized to Ca and the results are shown in Table B-1. Note that Ca concentrations are in ppm and all total trace elements are in ppb during the normalization. S1 had lower normalized concentrations of Ba, Mg, and Cu than D1, indicating that these species were depleted either by transport or removed during the wastewater treatment process. Cr and F^- did not have consistent concentration patterns between S1 and D1. S1 had consistently higher concentrations of the rest of the chemical species than D1, indicating these species are enriched by human input. S2 had exactly the same pattern as S1 when comparing its normalized concentrations to D2. S3C had lower normalized concentrations of Cu, Mg, SO_4^{2-} and NO_3^- than D3, indicating the depletion of these species during the transport from D3 to S3C. The rest of the chemical species are enriched in S3C by human inputs compared to D3.

The enrichment factors were calculated for each site and for each sampling season. Average enrichment factors for each source site during the whole year are shown in Table 3-11. The concentrations of some species in the three drinking water supplies were below the detection limit, which indicates that human input is the dominant source. These species are not included in Table 3-8. Ni, As, Na, and Cl^- are the most enriched species in Table 3-8.

Table 3-11
Average Enrichment Factors

	Average Enrichment Factor for S1/D1	Average Enrichment Factor for S2/D2	Average Enrichment Factor for S3C/D3
Sr	1.34	1.29	1.76
Mo	19.60	1.47	1.25
Ba	0.48	0.56	2.30
V	7.59	2.31	1.29
Ni	5.92	9.91	4.64
Co	2.13	24.13	2.66
Cu	0.14	0.05	0.32
Zn	6.07	24.48	0.69
As	21.75	48.20	32.64
Mg	0.41	0.85	0.48
Na	49.56	90.24	4.21
Cl^-	43.96	357.34	8.77
SO_4^{2-}	4.37	7.75	0.73

All values are reported to two decimal places, but no more than three figures are significant.

3.4.2 Evaluation of Potential Indicators by Three Requirements

The three requirements used in the evaluation of potential indicators were:

- Detectability
- Consistent Concentration Ratio of Potential Indicator to Phosphorus
- Uniqueness of Source Signature

3.4.2.1 Detectability

The estimated detection limits of the chemical species measured are listed in Table 3-12.

Table 3-12
Estimated Detection Limit of all Chemical Species

Chemical Species	Analytical Method	Detection Limit
Total phosphorus	Spectrophotometry, Standard Method 4500-P, ascorbic acid method after persulfate digestion	1 ppb
Dissolved reactive phosphorus	Spectrophotometry, Standard Method 4500-P, ascorbic acid method	1 ppb
F^-, Cl^-, Br^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, and NO_2^-	Ion Chromatography, Standard Method 4110	0.1 ppm,
Ca	ICP-OES, Standard Method 3120	10 ppb
Mg	ICP-OES, Standard Method 3120	30 ppb
K	ICP-OES, Standard Method 3120	100 ppb
Na	ICP-OES, Standard Method 3120	30 ppb
Sr	ICP-MS, Standard Method 3125	0.001 ppb
Mo	ICP-MS, Standard Method 3125	0.003 ppb
Cd	ICP-MS, Standard Method 3125	0.006 ppb
Sb	ICP-MS, Standard Method 3125	0.07 ppb
Ba	ICP-MS, Standard Method 3125	0.008 ppb
Pb	ICP-MS, Standard Method 3125	0.005 ppb
U	ICP-MS, Standard Method 3125	0.001 ppb
V	ICP-MS, Standard Method 3125	0.02 ppb
Cr	ICP-MS, Standard Method 3125	0.04 ppb
Ni	ICP-MS, Standard Method 3125	0.004 ppb
Co	ICP-MS, Standard Method 3125	0.002 ppb
Cu	ICP-MS, Standard Method 3125	0.003 ppb
Zn	ICP-MS, Standard Method 3125	0.017 ppb
As	ICP-MS, Standard Method 3125	0.025 ppb

Even after concentration with SPE, all four SOCs were not detected by the HPLC method with UV detection. ICP-MS does not have a good sensitivity for mercury. ICP does not have a good sensitivity for potassium. Nitrite (NO_2^-) was not detected in almost all samples. Thus these chemical species were excluded from the list of potential indicators. For the rest of the chemical species, current analytical methods were sensitive enough for quantification of the species in sources, receiving waters, and drinking water supplies.

3.4.2.2 Consistent Concentration Ratio of Potential Indicator to Phosphorus

The chemical indicators were evaluated with respect to their abilities to be consistent and reproducible indicators of the source of phosphorus. An important property for the evaluation is the indicator ratio (IR) as defined in Equation 3-2.

$$IR_{i,j,k} = C_{i,j,k} \Big/ TP_{j,k} \tag{3-2}$$

Equation 3-2 expresses the ratio of the concentration (C) of chemical species i to the total phosphorus (TP) concentration in source j during sampling time k. For a specific source, the best indicators will be those with consistent concentration ratios to phosphorus over time.

Since phosphorus concentrations are generally higher in sources and lower in receiving water bodies, potentially useful indicators should have a similar concentration distribution among the sources and receiving water bodies. Those chemical species with little variation among the sites can be excluded first; thus Cd, Sb, U, and Cr were excluded from the list of potential species. Pb was also excluded because of frequent detection failure by ICP-MS.

The consistency of indicator ratios is evaluated by plotting the concentrations of each potential indicator against their corresponding total phosphorus concentrations for all source sites. Scatter plots are shown in Appendix C. The scatter plots are based on the mean values of each season because the relative standard deviations of triplicate or duplicate samples are generally small. There are a total of four points for each species and each source (four seasons in a year). If the indicator ratios are constant, these four points will display a linear relationship that will pass through the origin. However, no species in this study displayed such a linear relationship.

The consistency of indicator ratio is further evaluated by calculating the indicator ratios according to Equation 3-2 based on the mean values of each season. Then the relative standard deviations of four indicator ratios of four seasons for each chemical species are calculated and shown in Table 3-13.

Table 3-13
Relative Standard Deviation of Indicator Ratios of Four Sampling Events

	Relative Standard Deviation of S1 (%)	Relative Standard Deviation of S2 (%)	Relative Standard Deviation of S3A (%)	Relative Standard Deviation of S3B (%)	Relative Standard Deviation of S3C (%)
Sr	21	98	100	120	99
Mo	61	111	107	178	99
Ba	12	99	98	81	61
V	48	89	87	175	136
Cr	90	143	126	175	105
Ni	88	110	103	122	64
Co	58	107	108	160	108
Cu	41	89	43	146	33
Zn	65	65	56	51	42
As	75	106	135	96	86
Ca	14	101	84	119	69
Mg	37	102	80	126	44
Na	45	100	62	135	31
F^-	49	64	121	200	153
Cl^-	51	102	81	91	39
SO_4^{2-}	30	108	171	86	111
Br^-	24	—	191	200	200
NO_3^-	110	53	176	182	161
PO_4^{3-}	200	81	41	122	37

The relative standard deviations of the indicator ratio of most chemical species in Table 3-13 are quite high. The species with the lowest relative standard deviations for indicator ratios across all sources are Ba, Cu, Zn, and Cl^-. For successful source apportionment of phosphorus, the potential indicators should have constant indicator ratios for all sources. However, both the scatter plots and relative standard deviation values demonstrate that there was no chemical

species that had constant indicator ratios in all sources. Thus these investigated chemical species may not be ideal indicators for phosphorus sources around Table Rock Lake.

One of the main reasons for the high variation of indicator ratios is the high variation of total phosphorus concentrations in sources over the whole year. Since the samples were collected seasonally in one year, the total phosphorus concentrations of a source site varied a lot among seasons due to differences in phosphorus inputs, especially for septic tanks. In addition, if the phosphorus removal operations of a WWTP were not stable, the total phosphorus concentrations of the effluents would vary considerably, such as at S2.

S1 had consistent total phosphorus concentrations over the year, indicating its phosphorus removal operations were stable. In addition, most chemical species were observed to have relatively consistent concentrations for S1 than the other source sites, indicating that the treatment processes of S1 function well and that the influent composition is stable over the year.

The utility of a chemical species as an indicator for a certain source can be examined by the relative standard deviation of its indicator ratio. For example, Br^- has a relative standard deviation of 24% and can be a potential indicator for S1. Here only those chemical species with indicator ratio relative standard deviations lower than 50% were considered.

- S1 had the following species with relatively constant ratio to total phosphorus: Sr, Ba, V, Cu, Ca, Mg, Na, F^-, Cl^-, SO_4^{2-}, and Br^-.
- S3A had the following species with relatively constant ratios to total phosphorus: Cu and PO_4^{3-}.
- S3C had the following species with relatively constant ratios to total phosphorus: Cu, Zn, Ca, Mg, Na, Cl^-, and PO_4^{3-}.
- S2 and S3B did not have any species with relatively constant ratios to total phosphorus because their total phosphorus concentrations had the greatest variations over the seasons.

Although their indicator ratios may have varied greatly, quite a few investigated chemical species were relatively constant during the whole year among sources. The ratios among them may indicate the sources of water input, but not necessarily of phosphorus loadings.

3.4.2.3 Uniqueness of Source Signature

The ideal indicator is a chemical species that is either only present or present at a much higher concentration in a certain source than in other types of sources. Br^- may be such an ideal indicator for S1. Only S1 had high concentrations of Br^- among all sources, and the concentrations of Br^- can be easily measured in the downstream site A1. S1 also had higher Mo concentrations than other sources, and Mo can potentially be used as an indicator.

The three septic systems had much higher Ni and Cu concentrations than the two WWTPs had, thus Ni and Cu can potentially be used as indicators of septic tank effluents. S1 and S2 had much higher concentrations of SO_4^{2-} than the three septic tanks. SO_4^{2-} can be used as a potential

indicator for WWTPs. A unique combination of several related variables (that is, factors extracted by PCA) can also be used as the signature of a certain source.

3.5 Summary and Recommendations for Future Work

Except for the analysis of SOCs by HPLC with UV detection, the analytical methods used in this project are sensitive and selective enough for the investigated chemical species. These methods can quantify the species in the source and receiving waters. To measure trace-level SOCs present in environmental water samples, a mass spectrometric detector is required. For successful source apportionment of phosphorus, the potential indicators should have constant indicator ratios among all sources. However, no chemical species were observed to have consistent concentration ratio to phosphorus among all source sites during the whole year.

The phosphorus concentrations varied greatly for the three septic systems and S2, which accounts for the high variation of these indicator ratios. However, some of the investigated chemical species were relatively constant during the whole year among sources. The ratios among them may indicate the sources of water input, but not necessarily of phosphorus loadings.

PCA confirmed that Br^- can be used as a unique indicator for S1. No other chemical species were observed that could be used as unique indicators of any other sources. However, Ni and Cu could potentially be used as indicators of the septic tank effluents, and SO_4^{2-} could be an indicator of WWTPs. PCA revealed the distribution patterns of the investigated chemical species among all sites and helped to interpret the experimental results.

Phosphorus concentration variation is a critical issue for evaluating indicator ratios over time. The four sampling campaigns of the current project were carried out in four different seasons in one year. The phosphorus concentrations of investigated source sites varied considerably, which made finding suitable indicators with constant indicator ratios difficult. If multiple sampling campaigns (and more sampling campaigns) for the source sites and receiving waters can be performed in a short period, it is likely that the concentrations of phosphorus and other chemical species will not vary as much as they do in one year. More campaigns can help identify suitable indicators more easily because the distribution pattern of chemical species among sites will be more stable. However, these indicators may only be useful for short periods of time and different seasons may have different indicators due to the variation of water quality. In addition, the more frequently the sites are sampled, the more accurate the PCA interpretation is as a statistical tool.

SOCs might be better indicators than the other chemical species investigated in the current project because human inputs are the main source of SOCs. Other chemical species investigated may have considerable geological sources, which makes the data interpretation more challenging. Even the use of bromide, which had high concentrations that were unique to the large wastewater treatment plant, as an indicator is limited by lack of knowledge of the specific sources of bromide to the wastewater treatment plant. Future work can identify these sources of bromide.

Table Rock Lake is a large lake and its water quality is generally quite good with respect to the chemical species. Because of the large volume of the lake, smaller discharges from septic systems can be rapidly diluted with water from other locations. Consequently, the imprint of the source profiles on the receiving water is difficult to observe. In contrast, the effect of S1 on the downstream site A1 and the three lake sites (A2, A3, and B) can be observed by current analytical methods. Thus in future project design, the scale difference between sources and receiving water is an important factor to be considered.

4 USE OF BACTERIOPHAGES TO ELUCIDATE THE SOURCE OF FECAL POLLUTION

4.1 Introduction

A variety of methods targeting biological macromolecules have been used to distinguish between fecal pollution of human and nonhuman origin (Elhmmali *et al.* 2000; Gilpin *et al.* 2003; Scott *et al.* 2002). Microbiological and molecular methods that include the culturing of bacteria originating from mammalian and bird intestines include:

- Fecal coliforms to fecal streptococci ratios (Geldreich and Kenner 1969)
- *Rhodococcus coprophilus* presence (Jagals *et al.* 1995; Mara and Oragui 1981)
- *Bifidobacterium* sp. presence (Mara and Oragui 1983; Resnick and Levin 1981)
- *Bacteroides* sp. presence (Kreader 1995)
- Repetitive DNA sequences of *Escherichia coli* (Dombek *et al.* 2000; Hassan *et al.* 2005)
- *E. coli* ribotypes (Carson *et al.* 2001; Carson *et al.* 2003; Parveen *et al.* 1999)
- Antibiotic resistant patterns (Harwood *et al.* 2000; Wiggins *et al.* 2003)

However, fecal source discrimination with bacterial culture-dependent methods are time-consuming and labor intensive, and they require extensive culture collections. In addition, it is now well accepted that a majority of bacteria residing in natural environments may be viable but non-culturable under laboratory conditions (Amann *et al.* 1995).

Detection of certain host-specific markers with molecular biology assays does not require the culturing of bacteria, and therefore is a more precise and rapid method of identifying sources of fecal pollution. Such molecular markers include specific nucleic acid sequences of bacteriophages (such as viruses of bacteria) infecting *Bacteroides fragilis* (Blanch *et al.* 2004; Puig *et al.* 1999; Puig *et al.* 2000; Tartera *et al.* 1989). However, the absence of *B. fragilis* phages in polluted waters and sewage in the United States and the inherent difficulty of performing the assay limit the usefulness of this marker (Havelaar *et al.* 1993; Jagals *et al.* 1995; Puig *et al.* 1999; Scott *et al.* 2002).

Fortunately, investigators have also reported that human and nonhuman feces contain different RNA coliphages (such as bacteriophages that infect *E. coli*), suggesting that these phages can be used to distinguish between human and nonhuman fecal sources of pollution (Cole *et al.* 2003; Havelaar and Hogeboom 1984; Luther and Fujioka 2004).

Coliphages have been typed into somatic and male-specific (F^+) groups, based on the mode of infection through either the cell wall or sex pilus, respectively. Work has targeted the F^+ coliphages as indicators, because certain subgroups of these phages are either highly associated with humans (such as groups II and III) or nonhumans (such as groups I and IV) (Scott *et al.* 2002).

To discriminate between fecal sources, F^+ RNA coliphages sampled from the environment must be typed. Historically, typing was performed according to serotyping assays after several viral isolation steps. These steps include:

1. Concentrating phages from environmental samples

2. Isolating phages with single or double agar layer plaque assay methods

3. Purifying and propagating phages in nutrient-rich broth

(Furuse *et al.* 1978; Grabow and Coubrough 1986; Griffin *et al.* 1999; Havelaar and Hogeboom 1984; Havelaar *et al.* 1993; Sinton *et al.* 1996; Sobsey and AWWA Research Foundation. 1995; Sobsey *et al.* 1990; US EPA 2000; US EPA 2001).

Methods of F^+ RNA coliphage serotyping have produced ambiguous results (Beekwilder *et al.* 1996). For this reason, genotyping of F^+ RNA coliphages was developed. The genotyping uses membrane hybridization with nucleic acid probes following viral isolation steps similar to those described for serotyping (Beekwilder *et al.* 1996; Hsu *et al.* 1995; Vinje *et al.* 2004). In addition, researchers have developed a method that uses reverse transcriptase polymerase chain reaction (RT-PCR) or PCR and a subsequent reverse-line blot hybridization technique for genotyping F^+ RNA or F^+ DNA coliphages, respectively (Vinje *et al.* 2004). Both serotyping and genotyping need to isolate viruses, which can be time-consuming.

The aim of this study was to target a series of bacteriophage species whose presence or absence act as indicators of fecal contamination. Primers for a RT-PCR technique to differentiate between fecal sources without the need for viral isolation and membrane hybridization were developed. A suite of three PCR primers specific for F^+ RNA coliphages was designed to discriminate between human and nonhuman fecal pollution after a propagation step. This method was tested with samples collected from Table Rock Lake. The study followed a coordinated plan of sampling and analysis of potential sources and lake water sites impacted by one particular type of source (source-rich surface waters – outlined in chapters 2 and 3).

The RT-PCR technique was used to identify bacteriophages. Single agar layer (SAL) or double agar layer (DAL) Petri dish techniques and a traditional most probable number (MPN) assay were used to quantify the bacteriophages. Because two bacterial hosts were used, two media and two growing environments were necessary:

- *B. fragilis* required anaerobic growth conditions and a DAL plating technique.
- *E. coli* necessitated a less nutrient-rich broth, an aerobic environment, and an SAL plaque assay technique.

Samples gathered for microbiological analysis were collected by two methods:

- Concentration with granular activated carbon (GAC)
- Direct sampling with membrane filtration to discard bacteria and debris

4.2 Materials and Methods

The following sections describe the samples used in this study and the laboratory methods for handling the samples.

4.2.1 Bacterial Strains and Bacteriophages

The American Type Culture Collection (ATCC) in Manassas, Virginia, supplied:

- *B. fragilis* strains RYC2056 and HSP40 (ATCC numbers 700786 and 51477, respectively)
- *E. coli* strain C-3000 (ATCC #15597)
- *B. fragilis* phages (ATCC #700786-B1 and ATCC #51477-B1)
- MS2 phage (ATCC #15597-B1)

Dr. Mark D. Sobsey of the University of North Carolina in Chapel Hill, North Carolina, supplied Bacteriophages GA and SP.

Bacteriophages MS2, GA, and SP were reference controls in all experiments for groups I, II, and IV, respectively. All ATCC cultures were grown following the directions provided by ATCC.

Phage stock concentrations were assessed using a spot assay viability test (single droplets of sample dispersed across an agar plate) using the DAL method for phages infecting *B. fragilis*, and the SAL method for F^+ RNA coliphages.

4.2.2 Chemicals and Enzymes

A majority of the chemicals used in this study were obtained from Sigma Chemical Company in St. Louis, Missouri. The media and their components (for example, peptone, tryptone, beef extract, yeast extract, agar powder, nutrient agar, and MacConkey agar) were obtained from Fisher Scientific in Chicago, Illinois. All enzymes used in this study were purchased from Promega in Madison, Wisconsin.

4.2.3 Sample Handling

Sampling locations and events for July 2004 through January 2005 were the same as described in Chapter 3. Additionally, a subset of samples was collected during May and August 2005. Sampling and onsite analyses were performed once per season to assess the effects of seasonal variation in source loadings and lake dynamics. Samples were collected and preserved onsite for

subsequent laboratory analysis; additional onsite measurements of water temperature, pH, dissolved oxygen, and conductivity were preformed in the field. 100 L samples from environmental waters and 50 L samples from wastewater treatment plants (WWTP) and septic tank sites were collected and concentrated onsite using GAC. In addition, 250 mL samples were collected from each site and filtered using 0.22 µm pore-size filters (Stericup, Millipore, Billerica, MA) and used for direct plaque assay by the SAL method and quantitative enumeration by the MPN method. All samples were processed within a week of collection.

4.2.4 Concentration of Samples

The samples were concentrated using the following method:

1. Coconut shell GAC, (General Carbon Corporation, Vacherie, Louisiana) was dry sterilized at 120 °C for two hours prior to viral concentration.

2. Water samples of 100 L or 50 L were collected from each site and adjusted to 0.5mM $AlCl_3$ and a pH of 5.5 with 1N HCl.

3. The samples were filtered at a rate of 2 L/min through 150 g of GAC in a PVC column (height: 43 cm; diameter: 11.5 cm).

4. The GAC was stored at 4 °C prior to further processing.

5. In the laboratory, adsorbed viruses were eluted from the GAC with 150 mL of urea-arginine phosphate buffer (UAPB) at pH 9.0.

6. Concentrated viruses in the eluent were precipitated by adding 1.8 mL of 1M $MgCl_2$ followed by centrifugation for 30 min at 3300 x g.

7. The resulting pellet was dissolved in 10 mL of McIlvaines buffer (pH 5.0) (Jothikumar *et al.* 1995).

4.2.5 Laboratory Methods for B. fragilis Analyses

The following sections describe the handling of *B. fragilis* for this study.

4.2.5.1 Double Agar Layer Bacteriophage Titer Assay for *B. fragilis*

The following steps were used in the DAL bacteriophage titer assay:

1. Bottom agar plates were prepared to assay each dilution of phage in triplicate.

2. 3-mL top agar tubes were prepared and kept at 45 ºC to avoid premature solidification of the agar.

3. The host bacteria were inoculated in 20 mL of Bacteroides phage recovery medium (BPRM) and grown at 37 ºC over night to achieve optimum growth.

4. Serial dilutions of the phage stock were made in BPRM and stored at 4 ºC until required (less than 3 months).

5. 1 mL of *B. fragilis* phage stock solution and 0.2 mL bacterial culture were added to each top agar tube, mixed by rolling, and poured onto a plate with bottom agar.

6. The suspension was spread evenly by tilting and rotating the plates.

7. The inverted plates were incubated in a BBL Gaspak (BD, Franklin Lakes, NJ) at 37 ºC and examined for plaque formation after a 24-hour incubation period.

8. For enumeration of environmental bacteriophages, 1 mL of concentrated water sample replaced the 1 mL of phage stock solution added to the melted top agar.

4.2.5.2 DNA Extraction From *B. fragilis*

DNA was extracted from *B. fragilis* using the following method:

1. Equal volumes of buffer saturated phenol and a concentrated phage sample (500 µL) were mixed thoroughly followed by centrifuging at 13,400 × g for 15 minutes.

2. The supernatant was collected and extracted again with an equal volume of buffer saturated phenol, mixed, and centrifuged.

3. The supernatant was double extracted with equal volumes of chloroform:isoamyl alcohol (1:1).

4. A 1/10 volume of 3 M $NaCH_3COOH$ (pH 5.2) was added to the final extraction supernatant.

5. The nucleic acids were precipitated with 2.5 volumes of ice-cold ethanol and incubated at –80 ºC for 2 to 4 hours.

6. After centrifugation (10 minutes, 9,300 × g) the precipitated nucleic acid pellet was washed with 100 µL of 70% ethanol, centrifuged for 10 minutes at 9,300 × g, decanted, and air-dried.

7. The washed nucleic acid was suspended in 10 µL of tris-EDTA buffer solution and stored at 20 ºC.

4.2.5.3 Nested PCR Method for *B. fragilis* Identification

A nested PCR method was used with two sets of oligonucleotide primers specific for *B. fragilis* phages adapted from a method developed by Puig *et al.* (2000). In this protocol, two separate PCR reactions are required for each sample. The first 50 µL reaction contained:

- 1.0 µL of template DNA
- 5.0 µL 10X PCR buffer
- 2.0 µL dNTP mix
- 4.0 µL of 0.1 µM external primers (Table 4-1)
- 2.5U *Taq* DNA polymerase

Table 4-1
Sequence of Primers for *B. fragilis* Coliphages

Primer	Sequence	Tm (°C)	Amplicon (bp)
External Forward	5'-GGGAAAGCACACAAGCG-3'	62	442
External Backward	5'-CAGAACATTAGTTTTACGG-3'	54	
Internal Forward	5'-GTGGCACGTGAACTTCCTTC-3'	62	328
Internal Backward	5'-CGTTTTGCATGGCATCCG-3'	60	

The following steps were used on the reaction mixture:

1. Denaturing at 94 ºC for 5 minutes
2. 30 cycles of denaturing at 94 ºC for 30 seconds
3. Annealing at 52 ºC for 30 seconds
4. Extension at 72 ºC for 1 minute
5. Extension at 72 ºC for 5 minutes to conclude the first amplification process

A second set of reactions were carried out with internal primers. These 50 µL reactions included:

- 1.0 µL of the external primer reaction product DNA
- 5.0 µL 10X PCR buffer
- 2.0 µL dNTP mix
- 6.0 µL of each 0.2 µM internal primer (Table 4-1)
- 2.5U *Taq* DNA polymerase

The prepared reaction tubes were amplified using the same protocol as the external primers with the exception of running 20 rather 30 cycles in the DNA thermocycler (Eppendorf, Mastercycler gradient, Hamburg, Germany).

4.2.6 Laboratory Methods for F^+ Coliphages

The following sections describe the handling of F^+ coliphages for this study.

4.2.6.1 Single Agar Layer Bacteriophage Titer Assay for F^+ RNA Coliphages

In the SAL bacteriophage titer assay, these steps were followed:

1. The host bacteria were inoculated in 5 mL of minimal media, which included:
   - 6.0 g Na_2HPO_4
   - 3.0 g KH_2PO_4
   - 100 mg Thiamine
   - 1.0 g NH_4Cl
   - 10 mL 10% glucose
   - 1.0 mL 1 M $MgSO_4$ in 1 L
2. The host bacteria were then incubated at 37 ºC for 22 to 24 hours and stored at 4 ºC for a maximum of four days.
3. The minimal media agar (100 mL) was prepared and cooled to 45 ºC.
4. 1 mL of host cells was added.
5. The mixture was poured onto Petri plates and allowed to solidify.
6. The plates were stored at 4 ºC for a maximum of four days.
7. Serial dilutions of phage stocks were made with 1X minimal media.
8. 0.5 mL of each dilution were spread evenly on prepared agar plates and allowed to absorb (10 to 15 minutes).
9. The plates were incubated overnight at 37 ºC and then examined for quantifiable plaque formation.

4.2.6.2 Single Agar Layer Assay for F^+ Coliphages in Concentrated Environmental Samples

Following elution from GAC, the concentrated samples were assessed for coliphage presence using an SAL method. Similar to the titer assay described in section 4.2.6.1:

1. 0.5 mL of concentrated sample was spread evenly across Petri dishes with an incorporated lawn of *E. coli* host cells in minimal media agar.
2. Triplicate plates of each sample were prepared.
3. The plates were allowed to absorb (10 to 15 minutes).

4. The plates were incubated overnight at 37 ºC, and observed the following morning for plaque formation.

4.2.6.3 Direct Plaque Assay for F^+ Coliphages in Environmental and Source Samples

For direct plaque assay of environmental and source samples:

1. 100 mL of 2X minimal media agar was prepared and cooled to 48 ºC to ensure the viability of the sample and host cultures.

2. 100 mL of each sample was filtered using 0.22 µm pore-size filters (no concentration) and brought to room temperature.

3. 2 mL of overnight host culture was added.

4. This sample and host culture was then added to a bottle of 2X minimal media agar and mixed gently to avoid the formation of bubbles.

5. The mixture was poured into ten 14-cm diameter disposable Petri dishes (without a bottom agar layer) and allowed to solidify.

6. The inverted plates were incubated overnight at 37 °C.

7. Plaques were counted (plaques were generally visible after 8 hours).

4.2.6.4 Most Probable Number Assay for F^+ Coliphages in Environmental and Source Samples

An MPN assay modified from *Standard Methods* 9221 C "Estimation of Bacterial Density" (Eaton *et al.* 1998) was used to quantify the number of bacteriophages present in the sample. In this method:

1. Three replicates for October and January sampling events and five replicates for May and August samples were aliquoted by a 10-fold dilution gradient into minimal media. The first set of dilution tubes included 10 mL of 2X media, 10 mL of filtered sample, and 100 µL of host bacteria.

2. Two additional dilution sets were prepared to include 1 mL or 0.1 mL of sample; the appropriate amount of 1X minimal media to achieve 10 mL, and 100 µL host bacteria. For the May and August sampling events, an additional dilution with 0.01 mL of sample was prepared.

3. All dilution sets were thoroughly mixed before being incubated at 37 ºC for 72 hours.

4. Following incubation, all tubes were analyzed for bacteriophage presence visually and through spot checks on prepared minimal media agar plates with incorporated host bacteria.

4.2.6.5 RNA Extraction from F^+ Coliphages

A TRI REAGENT LS (Sigma, St. Louis, Missouri) protocol for RNA isolation was followed according to the manufacturer's specifications. The resulting RNA pellet was suspended in 5 µL TE Buffer (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0). Extracted RNA was stored at –20 ºC before reverse transcription. RNA was extracted from both concentrated and filtered samples with a much higher concentration of extracted RNA gained from the filtered samples.

4.2.6.6 Primer Design for F^+ Coliphages

Multiple alignments were constructed using:

- ClustalX (1.81) software based on complete genomic sequences of F^+ RNA phages (MS2, GA, and SP)

Primer pairs were designed and evaluated using:

- Primer3 (Whitehead Institute for Biomedical Research, Cambridge, Massachusetts)
- NetPrimer (Premier Biosoft International, Palo Alto, California)
- OligoAnalyzer 3.0 (IDT-DNA, Coralville, Iowa) primer evaluation software

The final primer sequences were prepared using:

- Integrated DNA Technologies, INC (Coralville, Iowa)

4.2.6.7 Reverse Transcriptase—Polymerase Chain Reaction (RT-PCR)

Extracted RNA was transcribed into cDNA with reverse transcriptase:

1. A 10 µL solution with 1 µL of extracted sample and 1 µL of random primers was denatured for 10 minutes at 70 °C to release the virion RNA.
2. The solution was chilled on ice.
3. This template was subsequently added to a 10 µL solution composed of:
   - 100µM dNTP mix
   - 5U of placental RNase inhibitor
   - 1.5U of avian myeloblastosis virus reverse transcriptase
4. The suspension was transcribed at 45 °C for 30 minutes.

5. Subsequent PCR reactions were carried out in 25 µl reaction mixtures containing:
   - 1 µl of each enriched phage suspension
   - 1.5mM $MgCl_2$
   - 1.25U of *Taq* DNA polymerase
   - 20µg bovine serum album
   - 1 µL of each mix of forward and reverse primers (Table 4-2)
   - 100µM dNTP mix
   - 25µM betaine

Table 4-2
Sequence of Designed Primers for F^+ Coliphages

Coliphage	Primer	Sequence	Tm (°C)	Amplicon (bp)	Source
MS2	1F	5'-AATCTTCGTAAAACGTTCGTGTC-3'	53.7	204	Group I (nonhuman)
	1R	5'-GAGCCGTACCCACACCTTATAG-3'	56.8		
GA	6F	5'-CGTACTTAGCGGTATACTCAAGACC-3'	56.3	240	Group II (human)
	6R	5'-GTTTCCTGCATATAAGCATACCA-3'	52.9		
SP	2F	5'-TTAAACTAATTGGCGAGTCTGTACC-3'	54.9	236	Group IV (nonhuman)
	2R	5'-AACAGTGACTGCTTTATTTGAAGTG-3'	54.1		

6. The completed reaction mixture was heat-activated for 15 minutes at 95 °C.

7. 40 PCR cycles (denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute, and extension at 72 °C for 1 minute)

8. Final extension at 72 ºC for 10 minutes in a DNA thermocycler (Eppendorf, Mastercycler gradient, Hamburg, Germany)

4.3 Results

The purpose of this study was to develop a rapid molecular method to distinguish between human and animal sources of fecal pollution in samples gathered in the Table Rock Lake watershed. It was not possible to distinguish between human and nonhuman sources of fecal pollution with the developed assay, but fecal pollution was traced. In addition, seasonal effects on bacteriophage detection were shown.

4.3.1 Detection of Phages Infecting B. fragilis in Water

Viruses are present in environmental samples at very low concentrations as a result of mixing fecal pollution source waters with large bodies of natural waters. Detection of such low levels of viruses in environmental samples usually requires concentration of viruses from large volumes of water (ca. 100 L). A GAC-based UAPB elution method developed by Jothikkumar (1995) for the concentration of phages from environmental and source samples was used. After the sample concentration step, a molecular biology assay targeting bacteriophages from the animal and human gut bacterium, *B. fragilis*, was used. This assay, which was developed in Europe, included a DAL plaque enumeration and PCR identification through a nested PCR technique. Twelve samples were collected during the summer sampling event in July 2004 and analyzed for the presence of phages infecting *B. fragilis*. All samples were negative by both the plaque and the direct PCR assays. The positive control for bacteriophages from *B. fragilis* was positive (Figure 4-1).

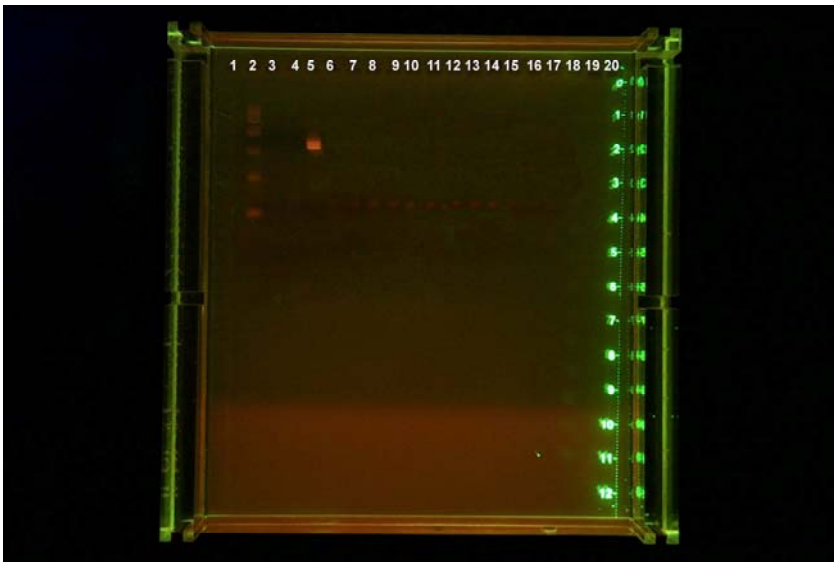

Figure 4-1
Agarose Gel Electrophoresis Showing the Amplification of *B. fragilis* Phage With Nested PCR Primers[6]

4.3.2 Detection of Phages Infecting B. fragilis in Fresh Sewage

Since no *B. fragilis* phages were found in the samples, targeting *B. fragilis* phages with American environmental samples—as opposed to European environmental samples—required confirmation. To evaluate the use of the GAC-UAPB-based nested PCR for detecting *B. fragilis*

[6] Lane 2—Molecular weight marker; Lane 3—Reagent control; Lane 4—Negative control; Lane 5—Positive control; Lane 6 to 17—DNA extracted using concentrated water samples collected from 12 different sampling sites at Table Rock Lake.

phages in American environmental samples, fresh sewage samples, with potentially the highest loads of bacteriophages specific for bacteria present in human guts, were collected from the Cold Water Creek wastewater treatment plant in Florissant, Missouri. Two L aliquots of raw sewage collected from the centralized wastewater treatment plant were concentrated by the GAC-UAPB method, followed by both DAL and PCR methods to enumerate and identify phages. The DAL plates observed after 24 hours of incubation showed distinct phage plaques on the cell lawns of *B. fragilis* strains HSP40 and RY C2056 with plaque size ranging from 1 mm to 4 mm in diameter (Figure 4-2).

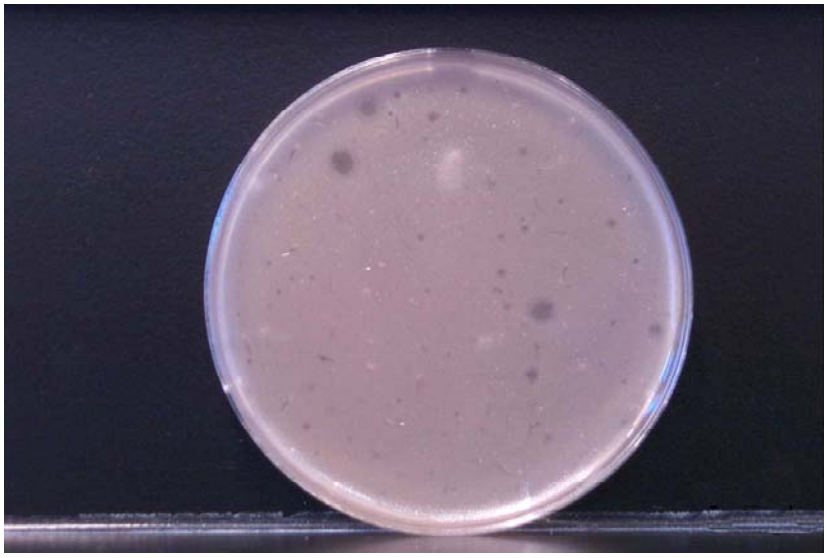

Figure 4-2
Plaques Produced by Phages in the Lawn of *B. fragilis* HSP40 by Direct Plaque Assay With a Concentrated Sewage Sample

Despite the observed phages on the DAL plates, the primers specific for human *B. fragilis* phages did not amplify the nucleic acid extracted from concentrated sewage samples. The positive control indicated a positive signal. Inhibitors present in the environmental samples could have impeded the primers from amplifying the DNA. Therefore, nucleic acid extracts of positive phage isolates were added to the extracted nucleic acid of environmental samples and the nested PCR assay was performed. The environmental samples did not negatively affect the PCR reaction, and therefore no inhibition was found.

A phage specificity test of the isolated phages from concentrated sewage samples was conducted, since the primers were reported to be specific for phages infecting *B. fragilis* HSP40. Phages isolated from sewage samples were tested for their specificity to two different strains of *B. fragilis* (HSP40 and RYC2056). Each of the *B. fragilis* phages (ATCC #700786-B1 and ATCC #51477-B1) infecting HSP40 were found to also infect RYC2056 and vice versa. Despite the presence of bacteriophages that can infect both HSP40 and RYC2056, the primers specific for European bacteriophages did not amplify the sample extracts. Therefore, nested primers specific to *B. fragilis* HSP40 are not useful for source discrimination in Missouri.

4.3.3 Detection of F+ RNA Coliphages in Sewage

Methods were applied to quantify and genotype F^+ RNA coliphages to elucidate the source of fecal pollution in water samples collected from the Table Rock Lake watershed. The shift from bacteriophages infecting *B. fragilis* to F^+ coliphages was, in part, driven by a much higher concentration of plaques in a raw sewage SAL plaque assay targeting F^+ coliphages compared to a DAL plaque assay targeting *B. fragilis* bacteriophages (Figure 4-3). The number of coliphages in the raw sewage indicated that F^+ coliphages were abundant and could be a promising indicator for the study.

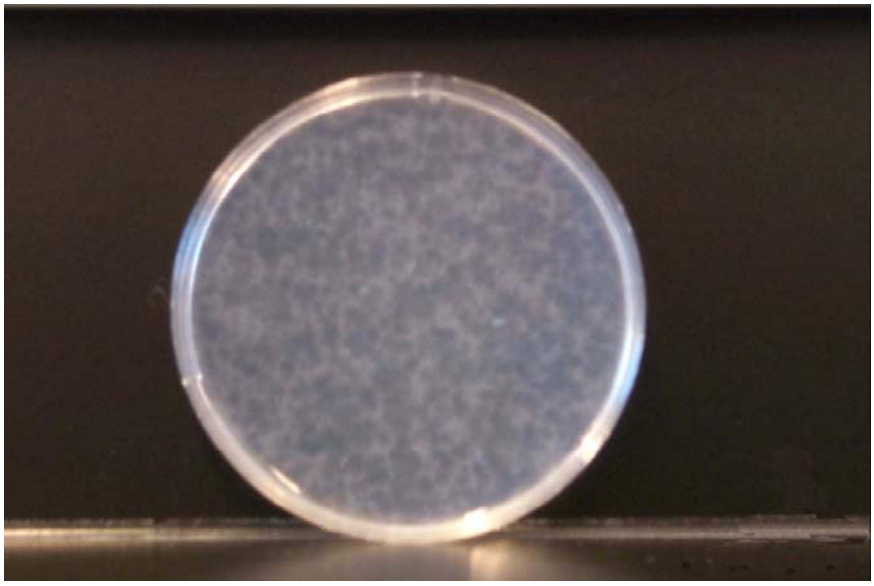

Figure 4-3
Plate Showing the F^+ RNA Coliphage Plaques on the Lawn of *E. coli*

4.3.4 Optimization of GAC-Based UAPB Method for Concentration of MS2 Using Spiked Water Samples

To determine the optimum pH for an efficient concentration of F^+ RNA coliphages, the recovery efficiency of MS2 (an F^+ RNA phage) was tested using water samples spiked with MS2 (100 PFU/2 L of sterilized deionized water) at five different pH values ranging from 4.0 to 6.5, followed by GAC-based UAPB methods for phage concentration. Virus recovery was assessed and the maximum recovery efficiency was 69% at a pH of 5.5 (Table 4-3).

Table 4-3
Optimum pH for the Concentration of MS2 Seeded in a 2 L Water Sample

Sample Number	pH	Spiked PFU/ 2 L	Percent Recovery
1	4.0	100	2%
2	4.5	100	4%
3	5.0	100	45%
4	5.5	100	69%
5	6.0	100	13%
6	6.5	100	5%

4.3.5 Enumeration and Identification of F^+ RNA Coliphages in Samples Collected During October 2004

Two environmental or source samples were collected at each sampling site:

- A 250 mL sample was filtered and stored in a sterile polypropylene bottle for direct enumeration of coliphages by the SAL method and an MPN test.
- A 100 L sample was concentrated using a GAC column for an enumeration plaque assay.

Four out of twelve filtered environmental samples were positive for F^+ coliphages for the October sampling event, while the two drinking water samples were negative (Table 4-4). Of the 12 GAC concentrated samples, the same four environmental samples were found to be positive for coliphages. Nucleic acid was extracted from each positive MPN tube; virus propagation amplified the number of phages originally present in the environmental sample ensuring adequate amounts of nucleic acid for RT-PCR identification techniques. The Springfield wastewater treatment plant (WWTP) upstream (C1), downstream (A1) and effluent (S1) samples tested positive for both MS2 and SP, groups I and IV nonhuman bacteriophages. The other positive sample, an Indian Point septic system (S3C), did not test positive with the coliphage primer sets used in this study, indicating the presence of other F^+ specific phages that did not correlate with the primer set used.

Table 4-4
Enumeration and Identification of F^+ RNA Coliphages From Samples Collected in October 2004[7]

	Location	Site Name	Direct MPN MPN/L	Direct Plaque Assay PFU/L	Concentrated Plaque Assay PFU/L	RT-PCR Technique
1	Downstream of Springfield WWTP	A1	750	600	1.2	MS2, SP
2	Upstream of Springfield WWTP	C1	430	380	0.2	MS2, SP
3	Downstream of Branson West WWTP	A2	0	0	0	—
4	Upstream of Branson West WWTP	C2	0	0	0	—
5	Lake Impacted by Septic Discharge, Indian point	A3	0	0	0	—
6	Lake Impacted by Chicken Waste, Kings River	A5	0	0	0	—
7	Less Developed Site, Piney Creek	B	0	0	0	—
8	Springfield WWTP Effluent	S1	40	10	0.2	MS2, SP
9	Branson West WWTP Effluent	S2	0	0	0	—
10	Septic System on Joe Bald	S3A	0	0	0	—
11	Septic System on Aunts Creek	S3B	0	0	0	—
12	Septic System on Indian Point	S3C	2400	900	3.2	Unamplified
13	Tap Water From Springfield	D1	0	0	NA	—
14	Tap Water From Indian Point	D3	0	0	NA	—

[7] Using MPN, plaque assay, and RT-PCR techniques

4.3.6 Enumeration and Identification of F^+ RNA Coliphages in Samples Collected During January 2005

During January 2005, samples were collected from 12 environmental and 2 drinking water samples from different taps of the domestic water supply. Filtered samples were collected from all sites and concentrated samples were collected only from the 12 environmental sites. Of the 250 mL filtered water samples collected, each of the environmental surface and source water samples analyzed for F^+ coliphages were positive, and the two drinking water samples were negative for F^+ coliphages by direct plaque assay (Table 4-5).

Table 4-5
Enumeration and Identification of F^+ RNA Coliphages From Samples Collected in January 2005

	Location	Site Name	Direct MPN MPN/L	Direct Plaque Assay PFU/L	Concentrated Plaque Assay PFU/L	RT-PCR Technique
1	Downstream of Springfield WWTP	A1	>11,000	550	0.1	MS2
2	Upstream of Springfield WWTP	C1	>11,000	1600	0.2	MS2
3	Downstream of Branson West WWTP	A2	>11,000	1580	0.2	MS2
4	Upstream of Branson West WWTP	C2	>11,000	1500	NA	MS2
5	Lake Impacted by Septic Discharge, Indian point	A3	11,000	4160	0	MS2
6	Lake Impacted by Chicken Waste, Kings River	A5	>11,000	4160	0.1	MS2
7	Less Developed Site, Piney Creek	B	11,000	1200	0.3	MS2
8	Springfield WWTP Effluent	S1	>11,000	790	2	MS2
9	Branson West WWTP Effluent	S2	>11,000	6890	3	MS2
10	Septic System on Joe Bald	S3A	>11,000	20	0	MS2

Table 4-5
Enumeration and Identification of F^+ RNA Coliphages From Samples Collected in January 2005[8] (Cont.)

	Location	Site Name	Direct MPN MPN/L	Direct Plaque Assay PFU/L	Concentrated Plaque Assay PFU/L	RT-PCR Technique
11	Septic System on Aunts Creek	S3B	>11,000	2500	4.6	MS2
12	Septic System on Indian Point	S3C	>11,000	370	0.6	MS2
13	Tap water From Springfield	D1	0	0	NA	—
14	Tap Water From Indian Point	D3	0	0	NA	—

The direct plaque assay resulted in an abundance of plaques (more than 50 per plate) with complete lysis observed in some plates. Similarly, the MPN results also showed more than 11,000 MPN/L of phages from the 12 environmental or source samples due to an overwhelming amount of phages and an insufficient dilution range. But both drinking water samples were negative.

Of the 12 concentrated samples (100 or 50 L volume), only 9 were positive for F^+ coliphages. The number of plaques (PFU/L) in the concentrated samples was lower than those obtained with the direct plaque assay. The low concentration of plaques in the GAC concentrated samples is attributed to a low recovery efficiency. Each of the 12 samples positive for F^+ with the direct samples were also positive for MS2 coliphage with RT-PCR analysis. MS2 coliphage is described in the literature as a nonhuman strain.

The levels of phages from the filtered samples were the highest (6,890 PFU/L) in the effluent from the Branson West WWTP (S2), while the phage concentration was considerably lower in the effluent from the Springfield WWTP (S1, 790 PFU/L). The lake samples contained high numbers of phages with 4,160 PFU/L for both of the sampling sites impacted downstream of the WWTPs (sites A3 and A5). Unanticipated high numbers (1,200 PFU/L) of F^+ RNA phages in the sampling location on the less developed site (B, the control site) correlated with unanticipated high total phosphorus levels of approximately 80 ppb. Though a comparison between total phosphorus levels and F^+ RNA phages did not show a statistical correlation, both independent indicators of fecal pollution suggested that mixing of lake water occurred during the late fall or early winter months.

[8] Using MPN, plaque assay, and RT-PCR techniques. NA=Not analyzed

Data from the US Geological Survey and the US Army Corps of Engineers (USACE 2005; USGS 2005) indicate that shifts in the water flow patterns in Table Rock Lake result from the opening and closing of various dams along the water body. These flow changes produce a backward flow of water from the body of the lake into individual lake fingers. This shift in water flow, or a natural upturn due to temperature gradients, may have caused fecal contamination in the study's control area.

4.3.7 Enumeration and Identification of F^+ RNA Coliphages in Samples Collected During May 2005

During May 2005, only 7 of the original 14 sites were sampled. Because this part of the study focused on the seasonal effects of viable F^+ coliphages as an indication of fecal pollution, samples from sewage treatment facilities, septic tanks, and drinking water sources were eliminated from the sample set. The eliminated source sites were expected to be the least affected by a change in seasonal temperatures, while the remaining seven sites were anticipated to be most dependent upon seasonal fluctuations. Therefore, only the seven environmental sites were analyzed for an additional two seasons to complete a four-season analysis.

At each of the seven sampling sites, 250-mL samples were collected with a sterile polypropylene bottle. Upon return to the lab, enumeration of coliphages by the SAL direct plaque and MPN assays was performed. Previous sampling events demonstrated the inferiority of the GAC column method compared to the direct filtration and sampling method. Thus the bulky concentration method was eliminated.

Three of the seven samples were positive for F^+ coliphages (Table 4-6).

Table 4-6
Enumeration and Identification of F^+ RNA Coliphages From Samples Collected in May 2005[9]

	Location	Site Name	Direct MPN MPN/L	Direct Plaque Assay PFU/L	RT-PCR Technique
1	Downstream of Springfield WWTP	A1	130	30	Unamplified
2	Upstream of Springfield WWTP	C1	216	30	GA
3	Downstream of Branson West WWTP	A2	0	0	—
4	Upstream of Branson West WWTP	C2	0	0	—

[9] Using MPN, plaque assay, and RT-PCR techniques.

Table 4-6
Enumeration and Identification of F^+ RNA Coliphages From Samples Collected in May 2005[9] (Cont.)

	Location	Site Name	Direct MPN MPN/L	Direct Plaque Assay PFU/L	RT-PCR Technique
5	Lake Impacted by Septic Discharge, Indian Point	A3	0	0	—
6	Lake Impacted by Chicken Waste, Kings River	A5	20	50	MS2
7	Less Developed Site, Piney Creek	B	0	0	—

RNA extraction and RT PCR techniques identified GA coliphages upstream of the Springfield WWTP (C1) and MS2 coliphages at the Kings River site (A5). Though the filtered sample taken downstream of the Springfield WWTP was positive for coliphage presence, the extracted RNA did not amplify with the primer sets.

4.3.8 Enumeration and Identification of F^+ RNA Coliphages in Samples Collected During August 2005

During the August 2005 sampling event, seven lake water samples were taken from the Table Rock Lake watershed; 250 mL samples were collected in sterile polypropylene bottles and filtered prior to further analysis. Direct plaque assay and MPN data from each water sample site yielded four locations positive for F^+ coliphages. Both upstream and downstream of the Springfield WWTP (C1 and A1), upstream of the Branson West WWTP (C2), and the lake sample on the Kings River (A5) contained F^+ RNA coliphages (Table 4-7).

Table 4-7
Enumeration and Identification of F^+ RNA Coliphages Collected From Samples in August 2005[10]

	Location	Site Name	Direct MPN MPN/L	Direct Plaque Assay PFU/L	RT-PCR Technique
1	Downstream of Springfield WWTP	A1	792	2480	Unamplified
2	Upstream of Springfield WWTP	C1	20	1990	GA
3	Downstream of Branson West WWTP	A2	0	0	—

[10] Using MPN, plaque assay, and RT-PCR techniques.

Table 4-7
Enumeration and Identification of F^+ RNA Coliphages Collected From Samples in August 2005[10] (Cont.)

	Location	Site Name	Direct MPN MPN/L	Direct Plaque Assay PFU/L	RT-PCR Technique
4	Upstream of Branson West WWTP	C2	2398	1470	Unamplified
5	Lake Impacted by Septic Discharge, Indian point	A3	0	0	—
6	Lake Impacted by Chicken Waste, Kings River	A5	4932	1090	Unamplified
7	Less Developed Site, Piney Creek	B	0	0	—

RT-PCR assays showed that upstream of the Springfield WWTP, GA F^+ RNA coliphages were present, indicating human fecal pollution in the water. The primer sets used in this study did not amplify the remaining three samples. Though a high concentration of coliphages were present—indicated by MPN and direct plaque assays—the RT-PCR was limited in finding additional F^+ RNA coliphage strain(s).

4.4 Discussion

The original experimental plan was designed to identify sources of human fecal pollution by enumeration and detection of phages specific for *B. fragilis* HSP40. This target phage was chosen because European studies have shown bacteriophages for *B. fragilis* in water impacted by human fecal pollution (Tartera *et al.* 1992; Tartera and Jofre 1987; Tartera *et al.* 1989). In addition, PCR-based detection of *B. fragilis* HSP40 phages is more sensitive than plaque assays, eliminating the need for phage propagation (Puig *et al.* 2000).

None of the samples collected during July 2004 tested by the GAC-UAPB nested PCR method were found to be positive for phages infecting *B. fragilis* HSP40. Further experimentation with a fresh raw sewage sample collected from a centralized wastewater treatment plant in Missouri, USA, was used to test the efficiency of the GAC-based UAPB method for the enumeration of phages infecting *B. fragilis*. The concentrated sample was analyzed by direct plate assay as well as the nested PCR method. *B. fragilis* phages at 40 PFU/mL of concentrated sewage sample were observed after 24 hours of incubation, but the primers were not able to amplify the DNA extracted from either the concentrated sample or from the propagated phages. Thus the primers that targeted *B. fragilis* HSP40 phages in Europe were unable to detect the *B. fragilis* HSP40 phages in American samples. The results found in this study are similar to the reports of other authors (Havelaar *et al.* 1993; Jagals *et al.* 1995; Puig *et al.* 1999). Instead of targeting bacteriophages of *B. fragilis*, coliphages were targeted for further studies.

F^+ RNA also has limitations as a fecal source identifier, despite promising correlations to viral concentrations in environmental waters (Cole *et al.* 2003). For example, the effect of temperature, pH, salt concentration, photo-oxidation, and chlorination on phage survival have been reported by several researchers (Maynard *et al.* 1999; Sinton *et al.* 1999). Among the factors that inactivate F^+ RNA bacteriophages, temperature and photo-oxidation are the critical inactivation factors in freshwater (Schaper *et al.* 2002). Thus seasonal changes likely impact the probability of detecting viable bacteriophages in the environment, as shown in Table 4-8.

Table 4-8
Samples Positive for F^+ Coliphages

Season	Number of positive samples out of number of samples taken
Fall 2004	4/14
Winter 2005	12/14
Spring 2005	3 /7
Summer 2005	4/7

The number of phages during the winter sampling event was the highest of all the samples. The number of F^+ coliphages surpassed the dilution scheme for the MPN test. An estimated concentration of more than 11,000 MPN/L was observed in each of the 12 source and environmental samples collected from the watershed during the winter. Both drinking water samples were negative.

Previous investigations have shown a significant seasonal variation in the concentration of F^+ RNA coliphages in environmental samples (Maynard *et al.* 1999; Schaper *et al.* 2002; Sinton *et al.* 1999). The inactivation rates of F^+ RNA were higher at warmer temperatures, which influenced the quantity of coliphage groups detected in environmental samples. In addition, Cole *et al.* (2003) have concluded that the resiliency of group I coliphages (such as MS2) is significantly higher than groups II, III, and IV.

In this study, the number of F^+ RNA detected was highest during cold weather sampling. Therefore, seasonal fluctuations have had an effect on the number and types of F^+ RNA phages found. Warm weather summer samples, however, also demonstrated high concentrations of F^+ coliphages. Concentration data and RT-PCR identification of the summer samples revealed that they contained substantial quantities of F^+ coliphages that were not amplified with the primer sets used in this study. That finding indicated that a more resilient and unknown type of coliphage may have been present in the summer than in the fall and spring.

This increase in detected F^+ coliphages may be due to an increase in F^+ DNA. The literature sites that F^+ DNA are more resistant to sunlight and warmer temperatures than F^+ RNA. This resistance makes them more readily detected in summer months (Cole *et al.* 2003; Vinje *et al.* 2004). The hydraulics of the lake may have contributed to the different types and concentrations of F^+ RNA coliphages over the seasons, as the opening of dams along the water body influenced the currents and mixing patterns within the system.

Environmental samples from locations that were the most impacted by fecal pollution—as determined by a multicriteria geospatial information systems (GIS, see Chapter 2)—yielded higher levels of F^+ RNA coliphages than the least impacted locations. Therefore, F^+ RNA coliphages can be considered an indicator of fecal pollution in the watershed. However, genotyping results did not show a correlation between the presence of human (GA) coliphages at human-impacted locations (sampling location A3) and the presence of nonhuman (MS2, SP) coliphages at nonhuman-impacted locations (sampling location A5).

Others have reported that genotyping data would ascertain if phages were from human or nonhuman origin (Cole *et al.* 2003; Havelaar and Hogeboom 1984; Luther and Fujioka 2004). In this study, genotyping was not successful in determining the source of pollution, primarily because bacteriophages that were described by others as "nonhuman" were present in the sources of human fecal pollution (Cole *et al.* 2003; Luther and Fujioka 2004; Vinje *et al.* 2004).

4.5 Summary and Recommendations for Future Work

The water samples collected during the July 2004 sampling event from the Table Rock Lake watershed were concentrated and analyzed through a GAC-UAPB nested PCR method for *B. fragilis*. All of the samples were negative for *B. fragilis* phages. Raw sewage samples from a local wastewater treatment facility were positive for *B. fragilis* by culture, but not by PCR. This finding indicates that primers specific for phages infecting *B. fragilis* in European watersheds are not useful for fecal source discrimination in US environmental waters.

Environmental samples were tested by two different assays:

- Direct filtration of 250-mL samples (filtered samples)
- Concentration of large samples (100/50 L) by the GAC-UAPB method (concentrated samples)

The direct filtration method gave more consistent results. Therefore, subsequent samples were tested by the direct filtration method only.

Samples collected during the four sampling events were analyzed for the presence of F^+ RNA coliphages by direct plating, MPN methods, and RT-PCR techniques. The data showed that F^+ phages can be used as a biological indicator for fecal pollution. Direct plaque assays and MPN studies showed significant concentrations of F^+ coliphages, but RT-PCR identification did not lead to a direct correlation between GIS human-impacted areas and group II RNA coliphages. There was also no direct correlation between nonhuman-impacted areas and group I and IV RNA coliphages.

In addition, F^+ coliphages that were supposed to correlate to nonhuman sources were found in the effluent of the sampled WWTPs, which were identified as human-only impacted sources. Therefore, using F^+ RNA coliphages to trace human versus nonhuman fecal pollution in environmental waters is not justifiable from the findings of this study. Since there was no

statistically significant correlation between phage numbers and total phosphorus concentrations, the phages cannot be used for phosphorus source apportionment.

Seasonal effects on bacteriophage presence were found in this study. Winter samples contained the highest concentration of coliphages. Fall and spring had the lowest concentration of coliphages. Samples from the summer showed higher F^+ coliphage concentrations than the spring and fall, but not as high as the winter. Coliphages isolated during the summer of 2005 were not amplified with the methods used in this study, and they remain an unknown strain. The higher concentrations during summer 2005 may be present due to a more robust F^+ DNA coliphage.

Additional studies are needed to elucidate the diversity of the coliphage population in the Table Rock Lake watershed. These studies could shed light on coliphages that may be used as an indicator of phosphorus pollution. To ascertain which coliphages are present in the watershed, all 22 known F^+ RNA coliphages in the four subgroups must be targeted instead of just the three coliphages used in this study (Regenmortel *et al.* 2000). Thus an additional 19 primer sets must be developed and tested.

In terms of methodology, development of a direct quantitative PCR (qPCR) assay of filtered environmental samples should have high priority. Such an assay would both identify and quantify the phages present, while eliminating the propagation step. This is advantageous because propagation adds time and effort, introduces the potential of contamination, and may have a lower sensitivity compared to qPCR.

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6 ACRONYMS AND ABBREVIATIONS

AFO	Animal Feeding Operation
ATCC	American Type Culture Collection
BOD	Biochemical Oxygen Demand
BPRM	Bacteroides Phage Recovery Medium
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DOC	Dissolved Organic Compound
DSF	Day Second Foot
GAC	Granular Activated Carbon
GC-MS	Gas Chromatography Mass Spectrometry
GIS	Geographic Information System
GPS	Geographic Positioning System
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography
HPLC-MS	High Performance Liquid Chromatography Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IR	Indicator Ratio
LMVP	Lakes of Missouri Volunteer Program

MPN	Most Probable Number
NPS	Nonpoint Source
NRCS	Natural Resources Conservation Service
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PFU	Plaque Forming Units
ppb	Parts Per Billion
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAL	Single Agar Layer
SOC	Synthetic Organic Compound
SPE	Solid Phase Extraction
TDS	Total Dissolved Solids
TMDL	Total Maximum Daily Load
TP	Total Phosphorus
TRLWQ	Table Rock Lake Water Quality, Incorporated
UAPB	Urea-Arginine Phosphate Buffer
US EPA	United States Environmental Protection Agency
USDA	United States Department of Agriculture
USGS	United States Geological Survey
UV	Ultraviolet
WWTP	Wastewater Treatment Plant

A WATER QUALITY SUMMARY

Table A-1
Site S1: Effluent of Springfield Southwest Wastewater Treatment Plant

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	7.20	>20.0	22	1250	111	—
Jul 2004	6.84	>20.0	28	1168	113	—
Oct 2004	7.31	>20.0	19	1450	94	53
Jan 2005	7.15	>20.0	3	1500	135	4

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	128.5	23.2	0.14	—	33.1	0	0.64	0.2	2.5	0.25	1.74	0.43	3.59	88.4	1.9
Jul 2004	140.2	40.1	0.39	0.75	38.1	0	1.52	0.0	2.4	0.52	4.29	0.80	5.13	69.5	21.2
Oct 2004	116.8	16.8	0.08	0.50	26.4	0	0.69	0.2	1.0	0.48	6.38	1.05	0.86	—	21.4
Jan 2005	100.6	3.6	0.01	0.26	38.0	0	0.00	0	0.8	0.05	0.86	0.44	1.87	21.5	9.0

Table A-1
Site S1: Effluent of Springfield Southwest Wastewater Treatment Plant (Cont.)

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	109.8	24.3	0.18	0.51	36.2	0	—	0.29	2.49	0	2.2	0.44	3.2	86.3	2.2
Jul 2004	100.1	28.8	0.35	0.54	28.8	0	1.09	0.16	1.86	0.32	2.8	0.59	1.6	47.3	15.0
Oct 2004	115.4	17.3	0	0.52	27.4	0	0.85	0.23	1.07	0.53	6.9	1.1	1.1	—	21.7
Jan 2005	101.8	2.8	0.05	0.27	44.5	0	0	0	0.90	0.27	1.4	0.59	3.2	25.2	12.0

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	67.6	3.9	6.7	134.5	0.78	152.0	0	98.6	38.6	48.1	0
Jul 2004	63.0	16.7	7.7	124.2	0.36	146.7	0.18	63.7	30.4	0	0.78
Oct 2004	66.5	—	8.1	137.7	0.39	187.1	0	72.0	24.1	6.1	0
Jan 2005	70.0	—	4.3	50.0	0.32	57.0	0	60.0	26.0	27.0	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-2
Site A1: James River Site, Downstream of Springfield Southwest Wastewater Treatment Plant

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	7.98	12.0	25	670	32	—
Jul 2004	7.16	5.8	33	660	80	—
Oct 2004	7.80	8.0	20	750	95	71
Jan 2005	7.28	10.4	5	550	38	24

Table A-2
Site A1: James River Site, Downstream of Springfield Southwest Wastewater Treatment Plant (Cont.)

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	73.0	4.6	0	—	59.1	0	0	0.4	1.5	0.3	0	0.2	2.2	31.3	0.9
Jul 2004	102.0	4.9	0.1	0.2	105.7	0	0.4	0.0	3.5	0.5	0.9	0.2	2.7	1.3	2.0
Oct 2004	86.1	9.1	0.2	0.4	50.3	0	0.3	0.3	1.4	0.4	2.9	0.4	1.7	—	4.9
Jan 2005	46.9	0.52	0	0.11	36.9	0	0	0	0.67	0.12	0.19	0.25	1.49	3.8	2.8

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	70.1	4.4	0.1	0.1	61.0	0	0	0.4	1.5	0	1.0	0.2	2.1	27.9	0.8
Jul 2004	74.0	3.2	0.1	0.1	79.8	0	0.7	0.4	2.9	0.5	1.3	0.4	2.3	1.6	1.6
Oct 2004	87.1	9.0	0.2	0.4	52.1	0	0.8	0.3	1.6	0.5	3.4	0.5	1.8	—	5.0
Jan 2005	49.7	0.53	0	0.11	38.7	0	0	0	0.85	0.21	0.60	0.38	1.70	3.4	2.9

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	68.7	8.4	7.0	26.3	0.36	35.2	0	24.1	4.26	11.5	0
Jul 2004	63.0	3.9	5.6	36.8	0.17	48.6	0	33.3	5.28	8.9	0.42
Oct 2004	66.4	—	5.4	38.0	0.16	43.9	0	30.0	4.93	7.0	0
Jan 2005	56.5	—	4.9	6.3	0.11	13.4	0	11.8	1.03	12.3	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-3
Site C1: James River Site, Upstream of Springfield Southwest Wastewater Treatment Plant

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	7.64	8.0	25	430	17	—
Jul 2004	6.75	5.6	29	464	34	31
Oct 2004	7.00	7.7	18	545	73	35
Jan 2005	7.74	11.6	7	510	35	27

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	62.1	2.0	0.02	—	120.3	0	0	0.38	1.5	0.27	0	0	2.6	60.6	0.83
Jul 2004	107.2	11.6	0.17	0.27	81.8	0	0.66	0.01	2.8	0.40	1.57	0.43	2.4	17.3	3.3
Oct 2004	84.2	6.54	0.20	0.21	80.3	0	0.24	0.40	1.5	0.38	0.84	0.21	2.2	—	1.9
Jan 2005	42.4	0.49	0	0.10	37.5	0	0	0	0.70	0.06	0.23	0.24	1.7	2.17	2.2

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	58.9	1.97	0.05	0.07	63.2	0	—	0.41	1.5	0	0.80	0.15	2.6	18.1	0.67
Jul 2004	92.0	9.39	0.16	0.23	68.8	0	1.06	0.35	2.5	0.52	1.9	0.50	2.1	15.3	2.6
Oct 2004	86.7	6.70	0.20	0.22	80.3	0	0.51	0.43	2.0	0.54	1.6	0.30	1.9	—	2.5
Jan 2005	41.9	0.44	0	0.10	39.2	0	0	0	0.90	0.12	0.59	0.33	1.9	2.0	2.1

Table A-3
Site C1: James River Site, Upstream of Springfield Southwest Wastewater Treatment Plant (Cont.)

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	59.4	10.2	8.2	8.7	0.05	16.1	0	12.0	0	4.67	0
Jul 2004	54.9	10.2	5.9	8.9	0.03	16.8	0	10.7	0	2.85	0.63
Oct 2004	68.8	—	4.3	11.8	0.05	15.4	0	12.5	0	6.7	0
Jan 2005	50.1	—	5.9	4.2	0.10	9.4	0	8.2	0	10.5	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-4
Site D1: Public Drinking Water Supply in Springfield, MO

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	6.67	10.4	13	505	9	—
Jul 2004	6.79	5.6	29	332	6	0
Oct 2004	7.22	2.9	21	375	3	1
Jan 2005	7.28	8.9	9	285	3	2

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	50.2	0.25	0.02	—	58.1	0	0.24	0.12	0.48	0.18	0	0	49.3	21.1	0.75
Jul 2004	43.9	3.94	0.08	0.04	44.5	0	0.96	0.10	0.10	0.12	0.08	0.09	11.2	1.46	0.19
Oct 2004	43.8	7.55	0.05	0	36.2	0	0.33	0.50	0.09	0.47	0.63	0.11	3.93	—	0.25
Jan 2005	36.6	3.12	0.00	0.08	26.0	0	0	0	0.15	0	0	0.21	5.22	5.01	0.63

Table A-4
Site D1: Public Drinking Water Supply in Springfield, MO (Cont.)

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	50.9	0.31	0.00	0.06	62.6	0	0	0.14	0.47	0	0.35	0.07	18.9	19.7	0.67
Jul 2004	50.0	4.49	0.09	0.04	50.3	0	1.05	0.33	0.07	0.12	0.20	0.10	13.0	1.9	0.22
Oct 2004	42.4	7.30	0.04	0.00	33.7	0	0.38	0.48	0.09	0.56	0.62	0.21	6.44	—	0.23
Jan 2005	37.1	3.15	0.00	0.08	26.0	0	0	0	0	0	0	0.20	5.59	3.8	0.68

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	63.1	7.34	6.02	9.40	1.19	22.1	0	10.3	0	4.03	0
Jul 2004	29.4	0.00	15.4	0.53	0.10	0.99	0	10.3	0	0.00	0
Oct 2004	32.1	—	17.0	1.1	0.17	1.04	0	10.1	0	0.00	0
Jan 2005	28.4	—	15.6	1.4	0.19	1.77	0	11.0	0	0.26	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-5
Site S2: Effluent of Branson West Wastewater Treatment Plant

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	6.68	5.5	23	1450	1240	—
Jul 2004	6.80	5.6	34	1208	111	—
Oct 2004	7.30	5.6	23	1300	60	7
Jan 2005	7.29	6.7	6	1065	318	288

Table A-5
Site S2: Effluent of Branson West Wastewater Treatment Plant (Cont.)

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	58.7	4.8	0.04	—	15.5	0	0.59	0.35	2.30	0.29	2.88	1.22	1.11	227.6	3.39
Jul 2004	54.4	4.5	0.20	0.45	5.98	0	0.65	0.07	0.51	0.22	5.08	3.49	1.47	95.6	23.7
Oct 2004	57.5	6.8	0.01	0.23	7.00	0	0.43	0.24	0.58	0.33	5.67	2.94	1.45	—	26.1
Jan 2005	53.1	5.6	0.02	0.49	7.20	0	0	0	0.36	0.00	2.47	1.33	2.62	178.1	23.7

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	56.8	5.2	0.08	0.70	5.01	0	0	0.50	2.27	0	3.33	1.07	1.59	207.4	3.13
Jul 2004	54.1	4.4	0.21	0.43	6.29	0	0.60	0.20	0.52	0.18	5.48	3.51	1.78	92.2	23.1
Oct 2004	57.5	6.8	0.01	0.23	7.00	0	0.43	0.24	0.58	0.33	5.67	2.94	1.45	—	26.1
Jan 2005	53.6	5.4	0.01	0.50	7.14	0	0	0	0.37	0.02	2.74	1.36	1.66	172.7	23.62

Table A-5
Site S2: Effluent of Branson West Wastewater Treatment Plant (Cont.)

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	37.7	25.5	18.8	177.0	0.16	264.0	0	51.4	0	2.89	5.80
Jul 2004	38.4	16.5	18.1	147.4	0.11	187.0	0	85.5	0	1.54	0.78
Oct 2004	41.7	—	19.5	133.0	0.07	194.2	0	82.7	0	0.74	0
Jan 2005	33.7	—	15.1	78.9	0.20	113.9	0	39.4	0	3.34	0.90

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-6
Site A2: Lake Site Near Aunts Creek, Downstream of Branson West Wastewater Treatment Plant

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	7.38	9.5	14	550	21	—
Jul 2004	7.53	7.6	31	228	14	9
Oct 2004	7.95	9.6	17	270	13	0
Jan 2005	7.22	10.7	7	355	15	1

Table A-6
Site A2: Lake Site Near Aunts Creek, Downstream of Branson West Wastewater Treatment Plant (Cont.)

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	43.2	0.61	0.01	—	31.9	0	0	0.22	0.62	0	0	0.13	0.53	4.58	0.54
Jul 2004	57.9	0.25	0.21	0.08	50.9	0	0.20	0.24	0.66	0.21	0.66	0.42	0.62	0.48	1.08
Oct 2004	36.3	4.32	0.00	0.06	20.7	0	0	0.23	0.82	0.29	0	0.16	0.18	—	1.39
Jan 2005	34.1	0.48	0	0.11	28.3	0	0	0	0.52	0	0.12	0.28	0.82	1.04	2.00

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	40.6	0.55	0	0.06	32.6	0	0	0.22	0.64	0	0.62	0.16	0.28	3.67	0.37
Jul 2004	58.1	0.22	0.20	0.08	52.1	0	0.34	0.24	0.66	0.22	0.79	0.41	0.79	0.39	1.14
Oct 2004	40.6	6.86	0.01	0.08	24.1	0	0.08	0.26	1.1	0.33	0	0.23	0.24	—	1.46
Jan 2005	34.2	0.48	0	0.11	28.1	0	0	0	0.66	0	0.45	0.33	1.16	1.44	2.09

Table A-6
Site A2: Lake Site Near Aunts Creek, Downstream of Branson West Wastewater Treatment Plant (Cont.)

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	44.5	7.6	7.6	7.2	0.07	11.3	0	9.7	0.53	2.12	0
Jul 2004	25.0	5.3	69	5.8	0.03	8.6	0	6.7	0.16	0	0.31
Oct 2004	26.2	—	7.4	7.1	0.04	9.9	0	7.2	0.34	0	0
Jan 2005	37.2	—	6.3	4.3	0.07	8.0	0	7.3	0.38	0	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-7
Site C2: A Small Creek Upstream of Branson West Wastewater Treatment Plant

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	7.15	9.5	13	445	10	—
Jul 2004	6.58	5.5	33	342	70	—
Oct 2004	7.06	6.5	19	550	13	9
Jan 2005	8.22	11.3	3	364	7	1

Dissolved Trace Element (ppb)															
Time	Sr	Mo	Cd	Sb	Ba	Hg	Pb	U	V	Cr	Ni	Co	Cu	Zn	As
Apr 2004	47.9	0.14	0.01	—	30.0	0	0	0.21	0.37	0.15	0	0	0.20	15.16	0.35
Jul 2004	42.5	0.91	0.12	0.16	30.0	0	0.25	0.05	1.25	0.25	1.24	0.16	3.52	3.42	2.01
Oct 2004	61.5	0.08	0.00	0.01	49.8	0	0.03	0.25	0.62	0.31	0	0.16	0.23	—	1.57
Jan 2005	44.4	0.01	0	0.09	27.4	0	0	0	0.21	0	0.10	0.24	1.18	0.73	2.02

Table A-7
Site C2: A Small Creek Upstream of Branson West Wastewater Treatment Plant (Cont.)

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	45.5	0.18	0	0.04	29.9	0	0	0.22	0.35	0	0.25	0.08	0.06	9.65	0.16
Jul 2004	36.8	0.69	0.13	0.11	30.7	0	0.37	0.30	1.20	0.25	0.99	0.43	0.81	8.39	0.89
Oct 2004	59.9	0.06	0	0	46.7	0	0	0.24	0.62	0	0	0.19	0.28	—	1.54
Jan 2005	46.7	0.01	0	0.09	27.5	0	0	0	0.23	0	0.10	0.25	1.31	0.32	2.14

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	57.8	5.2	5.2	4.2	0.07	8.0	0	9.3	0	2.9	0
Jul 2004	63.1	2.9	7.5	13.0	0.02	25.2	0	11.7	0	19.4	0
Oct 2004	76.7	—	7.1	8.1	0.03	17.7	0	13.2	0	3.2	0
Jan 2005	54.6	—	4.6	3.4	0.10	8.8	0	7.1	0	4.2	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method
C2 was dried out during July 2004 sampling and water samples were collected from a small pond remaining

Table A-8
Site D2: Public Drinking Water Supply in Branson West City, MO

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	6.72	5.9	16	370	4	—
Jul 2004	7.87	4.9	16	216	4	3
Oct 2004	7.36	7.0	18	330	4	2
Jan 2005	7.22	10.0	8	380	6	3

Table A-8
Site D2: Public Drinking Water Supply in Branson West City, MO (Cont.)

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	0	3.9	0.02	—	0.06	0	0.14	1.3	0.29	0.13	0	0	4.6	2.6	0.52
Jul 2004	44.5	3.4	0.14	0.07	17.2	0	2.44	1.2	0.19	0.16	0.19	0.07	78	7.9	0.44
Oct 2004	41.1	7.8	0.05	0	9.9	0	1.11	1.4	0.25	0.57	1.13	0.13	90	—	0.48
Jan 2005	47.2	2.7	0.04	0.10	12.9	0	0	1.1	0.24	0	0.30	0.22	14.1	5.3	0.88

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	0.18	4.13	0	0.03	0.15	0	0	1.7	0.27	0	0	0.03	6.9	2.52	0.34
Jul 2004	38.3	3.02	0.11	0.07	14.9	0	1.9	1.3	0.16	0.17	0.33	0.07	57.2	11.5	0.47
Oct 2004	39.8	7.27	0.07	0.01	10.0	0	1.3	1.4	0.26	0.48	1.15	0.14	90.5	—	0.36
Jan 2005	49.3	2.73	0.10	0.10	12.7	0	0.33	1.1	0.22	0	0.32	0.26	15.6	4.56	0.85

Table A-8
Site D2: Public Drinking Water Supply in Branson West City, MO (Cont.)

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	0	0	0	75.6	0.09	2.10	0	11.5	0	0	0
Jul 2004	36.9	32.6	19.8	0.90	0.02	1.49	0	10.5	0	0	0
Oct 2004	38.8	—	20.9	2.4	0.09	2.75	0	10.0	0	0.12	0
Jan 2005	36.7	—	20.6	1.4	0.14	1.89	0	11.5	0	0.20	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-9
Site S3C: Residential Septic Tank on Indian Point

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppm)
Apr 2004	7.46	0.8	20	3000	7.4 ppm	—
Jul 2004	7.10	1.1	25	2904	6.3 ppm	4.8 ppm
Oct 2004	7.14	<1.8	16	3050	7.5 ppm	6.7 ppm
Jan 2005	6.96	3.9	11	1010	1.2 ppm	976

Table A-9
Site S3C: Residential Septic Tank on Indian Point (Cont.)

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	64.0	0.68	0.06	—	28.6	0	0.62	0.86	0.32	0.51	1.6	0.19	13	35.5	3.30
Jul 2004	74.8	0.71	0.39	0.19	69.0	0	0.82	0.45	0.25	0.66	3.2	0.66	11	26.2	16.1
Oct 2004	68.9	13.7	0.06	0.62	26.6	0	0.55	0.67	0.71	1.02	3.6	0.56	18	—	35.8
Jan 2005	59.3	2.3	0	0.26	20.3	0	0	0	0.82	0.49	1.5	0.56	6.7	13.5	8.51

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	65.4	0.89	0.16	0.10	34.5	0	0	0.77	0.41	0	2.40	0.28	18.8	68.3	4.4
Jul 2004	68.4	0.96	0.48	0.19	62.6	0	1.01	0.97	0.31	0.80	4.08	0.76	19.6	65.8	17.7
Oct 2004	72.7	14.0	0.51	0.72	40.2	0	1.94	1.02	1.42	1.55	10.2	0.81	39.5	—	82.5
Jan 2005	58.1	2.2	0	0.26	20.4	0	0	0	1.08	0.64	2.1	0.59	4.0	23.0	8.2

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	59.5	48.0	38.2	290.6	0.50	307.4	0	16.4	0	1.3	14.0
Jul 2004	89.6	18.1	51.3	319.3	0.00	479.5	0	35.8	0	0	23.8
Oct 2004	51.6	—	37.0	240.4	0.00	282.6	0	9.1	0.64	0	19.2
Jan 2005	34.3	—	14.6	28.6	0.34	43.5	0	20.4	0	1.1	2.10

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-10
Site S3A: Residential Septic Tank on Joe Bald

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	6.60	0.8	17	6500	2.4 ppm	—
Jul 2004	6.22	1.3	9	4260	5.2 ppm	4.8 ppm
Oct 2004	7.27	<2.7	15	3800	1.3 ppm	0.8 ppm
Jan 2005	8.11	4.2	9	2060	6.1 ppm	4.9 ppm

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	409.0	1.1	0.02	—	98.8	0	0	0.39	0.18	0.53	7.89	10.1	0.80	26.2	13.5
Jul 2004	119.2	3.0	0.39	0.28	32.1	0	1.01	0.27	0.15	0.58	10.7	8.5	0.96	8.6	96.8
Oct 2004	174.6	24.8	0.03	0.30	19.3	0	0.14	1.45	1.04	0.68	10.7	6.1	0.56	—	68.1
Jan 2005	73.5	17.3	0.19	0.18	23.0	0	0	0	0.70	0.22	5.2	1.5	15.9	22.1	52.7

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	387.6	19.5	0.32	0.19	132.9	0	0	1.8	1.69	0	96.7	17.4	4.2	100.3	37.1
Jul 2004	57.2	2.84	0.45	0.24	18.6	0	1.31	0.82	0.18	0.76	4.56	0.76	31	199.7	1.79
Oct 2004	186.4	26.3	0.18	0.34	63.6	0	0.25	1.6	1.17	1.7	40.0	10.4	7.0	—	76.4
Jan 2005	67.6	21.5	0.41	0.24	30.8	0	0.30	1.0	1.38	2.5	24.2	2.4	26.4	65.9	29.1

Table A-10
Site S3A: Residential Septic Tank on Joe Bald (Cont.)

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	170.3	45.6	80.7	322.4	10.9	1376.4	0	8.70	1.41	2.40	12.2
Jul 2004	131.2	62.0	57.2	515.7	0	1049.4	0	16.6	0.11	0.07	15.9
Oct 2004	124.7	—	40.9	270.7	0	586.2	0	84.1	0	15.5	2.29
Jan 2005	32.0	—	11.6	191.5	0	249.91	0	36.21	0	2.28	16.2

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-11
Site S3B: Residential Septic Tank Near Aunts Creek

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	6.50	0.7	18	1130	9.6 ppm	—
Jul 2004	5.86	3.1	24	928	11.2 ppm	9.0 ppm
Oct 2004	6.67	<1.9	16	1200	13.0 ppm	6.2 ppm
Jan 2005	6.72	2.5	12	1010	8.3 ppm	5.2 ppm

Dissolved Trace Element (ppb)															
Time	Sr	Mo	Cd	Sb	Ba	Hg	Pb	U	V	Cr	Ni	Co	Cu	Zn	As
Apr 2004	48.75	2.61	0.10	—	10.8	0	0.27	0.46	0.13	0.37	2.1	0.00	28	122.2	1.0
Jul 2004	107.5	2.49	0.39	0.15	38.3	0	1.02	0.23	0.13	0.68	10.9	8.71	0.87	7.1	35.9
Oct 2004	49.0	14.2	0.12	0.22	13.6	0	0.36	0.57	0.59	1.19	2.4	0.53	52	—	4.8
Jan 2005	62.0	5.9	0.07	0.16	14.9	0	0	0	0.17	0.65	2.0	0.29	57.3	106.6	20.5

Table A-11
Site S3B: Residential Septic Tank Near Aunts Creek (Cont.)

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	47.6	3.46	0.18	0.14	12.6	0	0	0.78	0.14	0	2.9	0.17	37	176.7	0.89
Jul 2004	68.4	0.96	0.48	0.19	62.6	0	1.01	0.97	0.31	0.80	4.1	0.76	20	65.8	17.7
Oct 2004	48.9	14.6	0.13	0.20	10.0	0	0.50	0.93	0.65	1.24	2.7	0.88	60	—	6.4
Jan 2005	42.6	4.8	0.02	0.20	10.8	0	0	0	0.08	0.24	1.6	0.29	53.1	106.7	15.1

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	45.8	24.0	22.9	55.6	0.72	44.6	0	14.0	0	0.20	14.8
Jul 2004	55.1	8.0	19.8	54.6	0	42.8	0	1.4	0	0	30.5
Oct 2004	42.3	—	22.2	69.4	0	47.5	0	3.0	0.70	0	19.2
Jan 2005	37.5	—	20.4	55.7	17.4	70.6	0	6.0	0	1.09	23.5

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-12
Site A3: Lake Site Potentially Impacted by Septic Tank Discharge, Near Indian Point

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004 (Shallow)	7.47	9.7	14	350	5	—
Apr 2004 (Deep)	7.32	9.9	16	300	6	—
Jul 2004 (Shallow)	8.16	6.9	35	320	6	—
Jul 2004 (Deep)	8.26	6.5	36	284	6	3
Oct 2004 (Shallow)	7.73	8.6	21	365	6	0
Oct 2004 (Deep)	7.8	9	15.8	360	6	0
Jan 2005	7.80	9.2	11	450	5	1

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004 (Shallow)	37.3	0.69	0	—	30.3	0	0	0.18	0.36	0	0	0	0.49	5.1	0.59
Apr 2004 (Deep)	37.5	0.69	0	—	29.9	0	0	0.18	0.37	0	0	0	0.35	4.1	0.60
Jul 2004 (Shallow)	62.9	1.17	0.05	0.13	48.9	0	0.25	0.00	0.90	0.20	0.56	0.13	1.06	0.74	1.07
Oct 2004 (Shallow)	42.9	0.71	0	0.04	28.5	0	0.10	0.24	0.77	0.30	0	0.20	0.70	—	1.3
Oct 2004 (Deep)	39.1	6.15	0.13	0.14	28.1	0	0.12	0.36	1.29	0.33	1.77	0.24	0.50	—	3.0
Jan 2005 (Shallow)	36.2	0.52	0	0.12	29.1	0	0	0	0.34	0	0.21	0.23	1.48	0.51	2.3

Table A-12
Site A3: Lake Site Potentially Impacted by Septic Tank Discharge, Near Indian Point (Cont.)

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004 (Shallow)	34.8	0.73	0	0.06	29.7	0	0	0.20	0.33	0	0.45	0.07	0.25	5.25	0.38
Apr 2004 (Deep)	34.0	0.74	0	0.06	29.6	0	0	0.20	0.34	0	0.45	0.07	0.22	4.73	0.38
Jul 2004 (Shallow)	39.4	0.72	0.05	0.09	29.9	0	0.23	0.08	0.61	0.18	0.42	0.12	0.74	0	0.82
Jul 2004 (Deep)	41.6	0.77	0.05	0.10	32.4	0	0.17	0.05	0.59	0.17	0.40	0.12	0.67	0	0.80
Oct 2004 (Shallow)	41.3	0.71	0	0.04	28.3	0	0	0.26	0.95	0.25	0	0.17	0.31	—	0
Oct 2004 (Deep)	41.5	6.58	0.24	0.14	29.3	0	0.12	0.37	1.10	0.30	6.23	0.24	2.30	—	0
Jan 2005 (Shallow)	32.8	0.50	0	0.11	29.0	0	0	0	0.38	0	0.20	0.24	0.64	0.47	2.3

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004 (Shallow)	33.3	7.3	6.2	5.9	0.06	8.9	0	9.3	0.52	1.00	0
Apr 2004 (Deep)	34.0	6.4	6.4	6.0	0.06	8.9	0	9.1	0.50	0.95	0
Jul 2004 (Shallow)	24.6	0.94	5.4	5.0	0.03	8.6	0	7.3	0.20	0	0
Jul 2004 (Deep)	28.0	3.3	6.4	6.1	0.03	8.8	0	7.4	0.21	0	0
Oct 2004 (Shallow)	32.3	—	7.2	5.8	0.03	8.0	0	7.0	0.16	0.03	0
Oct 2004 (Deep)	32.5	—	7.2	6.1	0.09	8.1	0	6.9	0.16	0.13	0
Jan 2005 (Shallow)	56.5	—	4.9	6.3	0.06	7.8	0	33.3	0.28	1.40	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-13
Site D3: Well Water on Indian Point

Time	pH	DO (ppm)	Temperature (ºC)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	6.55	5.3	19	995	9	—
Jul 2004	6.36	4.6	26	936	7	5
Oct 2004	6.78	6.2	19	1190	9	4
Jan 2005	6.96	6.0	13	905	9	8

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	27.3	0.90	0.01	—	12.4	0	0.93	1.09	0.32	2.16	1.3	0.15	36.7	39.0	0.46
Jul 2004	32.8	1.07	0.12	0.07	14.3	0	2.58	0.84	0.30	0.25	0.71	0.16	35.5	29.3	0.85
Oct 2004	25.3	4.89	0.05	0.01	12.2	0	1.98	0.92	0.58	0.52	0.99	0.20	28.5	—	0.87
Jan 2005	0	0.31	0	0.10	0.18	0	0	0	0.34	0	1.9	0.29	9.0	7.7	5.7

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	29.5	0.97	0.00	0.05	14.0	0	0	1.2	0.31	0.00	0.89	0.13	60.0	101.9	0.33
Jul 2004	32.7	1.0	0.10	0.06	14.2	0	4.2	0.94	0.29	0.20	0.68	0.16	55.8	34.3	0.82
Oct 2004	24.2	4.7	0.05	0.02	11.6	0	4.4	1.1	0.53	0.66	0.98	0.17	63.4	—	0.93
Jan 2005	0	0.27	0	0.10	0.09	0	0	0	0.33	0	1.7	0.31	10.4	6.7	5.4

Table A-13
Site D3: Well Water on Indian Point (Cont.)

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	45.4	72.6	52.7	63.9	0.10	28.3	0	20.0	0	10.3	0
Jul 2004	40.5	97.3	55.8	51.5	0	25.7	0	15.7	0	6.68	0
Oct 2004	45.3	—	69.6	33.3	0	25.9	0	14.9	0	5.86	0
Jan 2005	0.3	—	0	178.8	0.12	28.9	0	16.0	0	12.0	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-14
Site A5: Kings River Site, Potentially Impacted by Animal Feeding Operations

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004	8.22	10.5	16	315	54	—
Jul 2004 (shallow)	6.48	7.2	34	244	57	—
Jul 2004 (deep)	6.61	6.1	35	228	60	—
Oct 2004	6.82	7.9	16	555	52	33
Jan 2005	7.10	10.2	9	239	20	16

Table A-14
Site A5: Kings River Site, Potentially Impacted by Animal Feeding Operations (Cont.)

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	31.0	0.30	0	—	22.9	0	0	0.19	0.64	0	0	0.14	0.37	6.2	0.54
Jul 2004 (shallow)	43.6	6.69	0.20	0.21	32.8	0	0.16	0.06	1.11	0.16	0.61	0.17	0.80	1.2	1.4
Jul 2004 (Deep)	48.7	7.32	0.21	0.22	35.8	0	0.22	-0.01	1.12	0.24	0.59	0.15	0.98	1.0	1.3
Oct 2004	46.4	1.84	0	0.10	43.8	0	0.13	0.60	1.38	0.30	0.61	0.29	0.74	—	1.8
Jan 2005	25.9	0.04	0	0.10	23.7	0	0	0	0.29	0	0	0.28	0.65	0.57	1.2

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004	29.6	0.34	0	0.07	24.9	0	0	0.20	0.63	0	0.54	0.18	0.27	5.07	0.35
Jul 2004 (shallow)	37.3	5.64	0.19	0.19	29.3	0	0.43	0.23	1.13	0.23	1.11	0.29	0.73	0.81	1.1
Jul 2004 (Deep)	38.1	0.37	0.17	0.15	29.1	0	0.59	0.16	1.05	0.21	0.84	0.21	1.06	0.58	0.99
Oct 2004	45.8	1.71	0	0.10	44.7	0	0.33	0.61	1.57	0.38	1.37	0.47	0.43	—	1.8
Jan 2005	25.9	0.04	0	0.10	22.7	0	0	0	0.33	0	0.09	0.28	0.49	0.57	1.2

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004	33.7	12.1	9.5	4.7	0.04	5.3	0	8.0	0	0.53	0
Jul 2004 shallow)	38.6	2.0	8.0	2.8	0.02	3.8	0	4.9	0	2.3	0.22
Jul 2004 (Deep)	40.3	2.2	8.0	2.5	0.02	3.7	0	4.8	0	2.5	0
Oct 2004	12.5	—	4.7	4.5	0.02	12.1	0	8.2	0	0	0.06
Jan 2005	30.2	—	8.0	2.5	0.04	4.6	0	6.4	0	7.3	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

Table A-15
Site B: Less Developed Control Lake Site, Near Piney Creek

Time	pH	DO (ppm)	Temperature (°C)	Conductivity (µS/cm)	Total Phosphorus (ppb)	Dissolved Reactive Phosphorus (ppb)
Apr 2004 (Shallow)	8.13	10.9	17	440	—	—
Apr 2004 (Deep)	8.25	11.0	14	363	25	—
Jul 2004 (Shallow)	7.97	8.8	36	192	12	—
Jul 2004 (Deep)	7.82	6.0	40	256	10	6
Oct 2004 (Shallow)	7.50	8.8	16	340	14	0
Oct 2004 (Deep)	7.66	8.4	18	315	11	0
Jan 2005 (Shallow)	7.32	9.5	8	335	82	42

Dissolved Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004 (Shallow)	44.1	0.86	0.01	—	37.0	0	0	0.25	0.96	0.18	0	0.12	0.68	2.4	0.55
Apr 2004 (Deep)	45.6	0.85	0.01	—	38.5	0	0	0.26	0.91	0.17	0	0.12	0.73	3.5	0.54
Jul 2004 (Shallow)	34.7	0.95	0.13	0.14	19.6	0	0.16	0.01	0.99	0.16	0.46	0.14	0.76	7.7	1.61
Jul 2004 (Deep)	41.7	1.3	0.15	0.18	25.8	0	0.21	0.01	1.14	0.12	0.48	0.17	0.99	0.88	1.57
Oct 2004 (Shallow)	46.8	3.3	0.05	0.12	25.1	0	0.08	0.38	0.97	0.78	6.78	0.24	4.79	—	—
Oct 2004 (Deep)	47.7	2.2	0	0.10	16.3	0	0.01	0.37	0.95	0.30	0.44	0.30	0.10	—	0.69
Jan 2005	33.2	0.28	0	0.11	32.4	0	0	0	0.74	0.06	0.19	0.25	0.99	3.3	2.1

Table A-15
Site B: Less Developed Control Lake Site, Near Piney Creek (Cont.)

Total Trace Element (ppb)															
Time	**Sr**	**Mo**	**Cd**	**Sb**	**Ba**	**Hg**	**Pb**	**U**	**V**	**Cr**	**Ni**	**Co**	**Cu**	**Zn**	**As**
Apr 2004 (Shallow)	43.1	0.89	0	0.07	37.9	0	0	0.27	0.93	0.00	0.55	0.14	0.50	1.2	0.37
Apr 2004 (Deep)	42.7	0.89	0	0.07	37.2	0	0	0.27	0.94	0.00	0.55	0.15	0.49	1.1	0.37
Jul 2004 (Shallow)	38.7	1.01	0.16	0.15	40.9	0	0.24	0.16	1.13	0.18	0.56	0.18	0.62	6.6	1.5
Jul 2004 (Deep)	39.1	1.06	0.17	0.16	28.5	0	0.22	0.16	1.22	0.12	0.56	0.19	0.63	0.47	1.8
Oct 2004 (Shallow)	48.3	3.16	0	0.10	32.6	0	0.15	0.37	1.13	0.29	0.85	0.31	0.24	—	0.82
Oct 2004 (Deep)	69.9	6.13	0	0.45	37.6	0	0.59	0.34	1.14	0.27	0.87	0.31	0.18	—	1.1
Jan 2005	33.8	0.23	0	0.11	34.3	0	0	0	0.98	0.11	0.78	0.38	0.96	2.7	2.3

Major Elements (ppm)					Major Anions (ppm)						
Time	Ca	K	Mg	Na	F^-	Cl^-	NO_2^-	SO_4^{2-}	Br^-	NO_3^-	PO_4^{3-}
Apr 2004 (Shallow)	48.4	7.2	7.4	7.2	0.05	11.9	0	9.8	0.71	5.8	0
Apr 2004 (Deep)	47.1	6.9	6.9	7.1	0.06	12.3	0	9.9	0.76	6.0	0
Jul 2004 (Shallow)	25.1	2.3	6.0	7.1	0.03	10.5	0	7.0	0.39	0	0
Jul 2004 (Deep)	25.9	1.6	6.0	7.1	0.03	10.5	0	7.0	0.38	0	0
Oct 2004 (Shallow)	27.9	—	6.7	10.9	0.03	14.5	0	8.8	0.84	0.01	0
Oct 2004 (Deep)	28.1	—	6.9	10.7	0.02	14.4	0	8.8	0.82	0	0
Jan 2005 (Shallow)	41.9	—	4.9	3.3	0.05	7.6	0	6.6	—	9.4	0

— means data not available due to analytical equipment failure
0 means concentration is below the detection limit of the analytical method

B NORMALIZED CONCENTRATIONS

Table B-1
Normalization of Chemical Species to Ca for Related Source Site and Drinking Water Supply

Site	Sr	Mo	Cd	Sb	Ba	V51	Cr	Ni	Co	Cu
S1, Apr 2004	1.625	0.359	0.003	0.008	0.536	0.037	0	0.032	0.006	0.047
D1, Apr 2004	0.807	0.005	0.000	0	0.991	0.008	0	0.006	0	0.299
S1, July 2004	1.588	0.457	0.006	0.009	0.457	0.029	0.005	0.045	0.009	0.025
D1, July 2004	1.701	0.153	0.003	0	1.711	0.002	0.004	0.007	0.004	0.443
S1, Oct 2004	1.736	0.261	0.000	0.008	0.413	0.016	0.008	0.103	0.017	0.017
D1, Oct 2004	1.320	0.227	0.001	0	1.048	0.003	0.017	0.019	0.007	0.200
S1, Jan 2005	1.454	0.039	0.001	0.004	0.636	0.013	0.004	0.020	0.008	0.046
D1, Jan 2005	1.307	0.111	0.000	0.003	0.914	0	0	0	0.007	0.197
S2, July 2004	1.406	0.115	0.005	0.011	0.164	0.013	0.005	0.142	0.091	0.046
D2, July 2004	1.040	0.082	0.003	0.000	0.403	0.004	0.005	0.009	0.002	1.551
S2, Oct 2004	1.379	0.162	0	0.006	0.168	0.014	0.008	0.136	0.071	0.035
D2, Oct 2004	1.026	0.188	0	0.000	0.257	0.007	0.012	0.030	0.004	2.335
S2, Jan 2005	1.592	0.161	0	0.015	0.212	0.011	0.001	0.081	0.040	0.049
D2, Jan 2005	1.343	0.074	0.003	0.003	0.345	0.006	0.000	0.009	0.007	0.426
S3C, Apr 2004	1.099	0.015	0.003	0.002	0.579	0.007	0.000	0.040	0.005	0.317
D3, Apr 2004	0.649	0.021	0.000	0.000	0.309	0.007	0.000	0.020	0.003	1.322
S3C, July 2004	0.763	0.011	0.005	0.002	0.698	0.003	0.009	0.046	0.009	0.219
D3, July 2004	0.806	0.025	0.002	0.000	0.351	0.007	0.005	0.017	0.004	1.376
S3C, Oct 2004	1.410	0.271	0.010	0.014	0.779	0.028	0.030	0.197	0.016	0.767
D3, Oct 2004	0.534	0.103	0.000	0.000	0.257	0.012	0.015	0.022	0.004	1.399

Table B-1
Normalization of Chemical Species to Ca for Related Source Site and Drinking Water Supply (Cont.)

Site	Zn	As	Mg	Na	F^-	Cl^-	SO_4^{2-}	Br^-	NO_3^-
S1, Apr 2004	1.276	0.032	0.099	1.990	0.012	2.249	1.459	0.571	0.712
D1, Apr 2004	0.315	0.011	0.095	0.149	0.019	0.350	0.163	0	0.064
S1, July 2004	0.751	0.238	0.123	1.971	0.006	2.329	1.011	0.482	0
D1, July 2004	0.066	0.008	0.522	0.018	0.003	0.034	0.351	0	0
S1, Oct 2004	—	0.327	0.123	2.073	0.006	2.816	1.084	0.363	0.091
D1, Oct 2004	—	0.007	0.529	0.034	0.005	0.032	0.315	0	0
S1, Jan 2005	0.360	0.171	0.061	0.714	0.005	0.814	0.857	0.371	0.386
D1, Jan 2005	0.133	0.024	0.549	0.049	0.007	0.062	0.386	0	0.009
S2, July 2004	2.397	0.600	0.472	3.833	0.003	4.862	2.223	0	0.040
D2, July 2004	0.311	0.013	0.539	0.024	0.000	0.040	0.285	0	0.000
S2, Oct 2004	—	0.625	0.468	3.192	0.002	4.660	1.985	0	0.018
D2, Oct 2004	—	0.009	0.538	0.062	0.002	0.071	0.258	0	0.003
S2, Jan 2005	5.124	0.701	0.448	2.341	0.006	3.380	1.169	0	0.099
D2, Jan 2005	0.124	0.023	0.561	0.038	0.038	0.004	0	0.312	0.000
S3C, Apr 2004	1.149	0.074	0.642	4.884	0.008	5.166	0.276	0	0.022
D3, Apr 2004	2.245	0.007	1.161	1.407	0.002	0.623	0.441	0	0.227
S3C, July 2004	0.734	0.197	0.573	3.562	0	5.350	0.399	0	0
D3, July 2004	0.847	0.020	1.376	1.270	0	0.634	0.387	0	0.165
S3C, Oct 2004	—	1.600	0.717	4.661	0	5.479	0.176	0	0
D3, Oct 2004	—	0.021	1.535	0.734	0	0.572	0.330	0	0.129

C SCATTER PLOTS

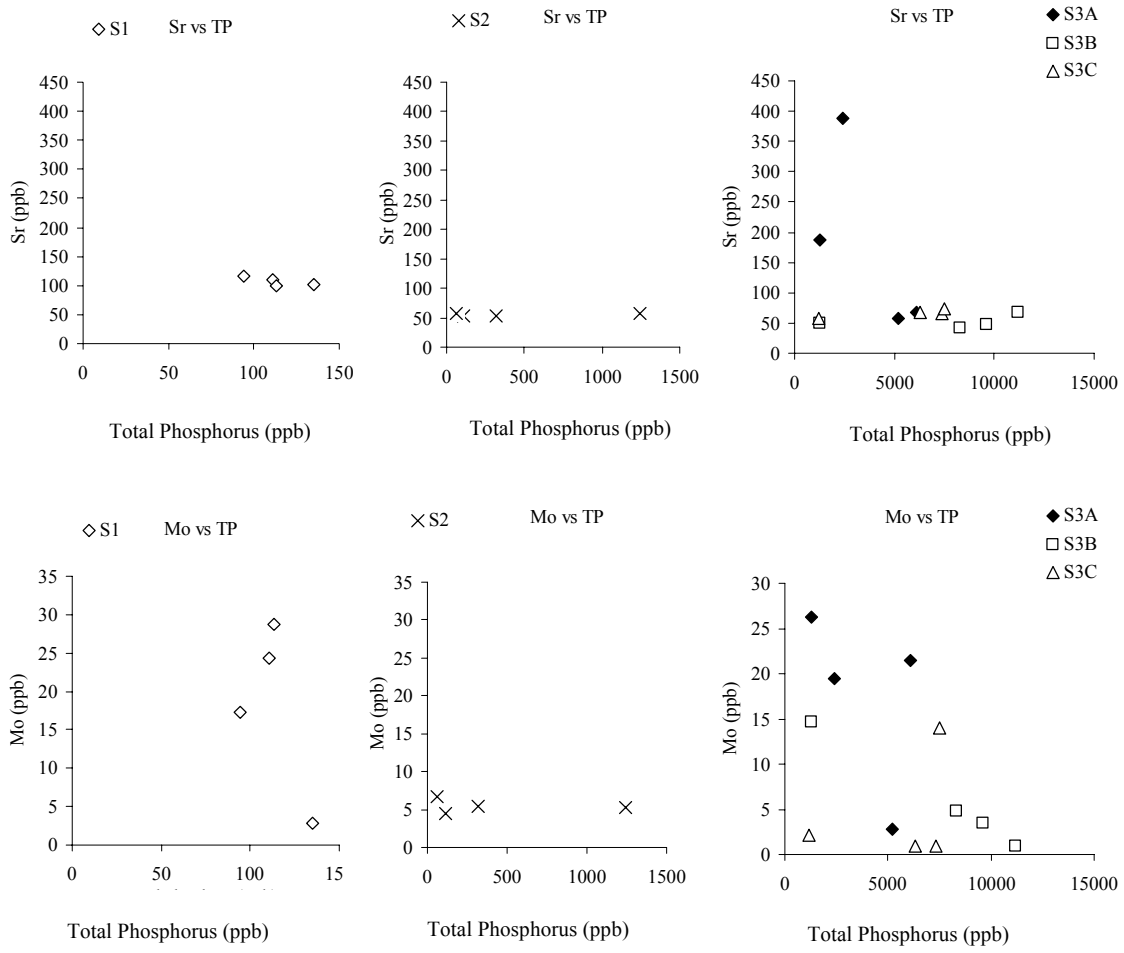

Figure C-1
Scatter Plots of Chemical Species Versus Corresponding Total Phosphorus for Five Source Sites

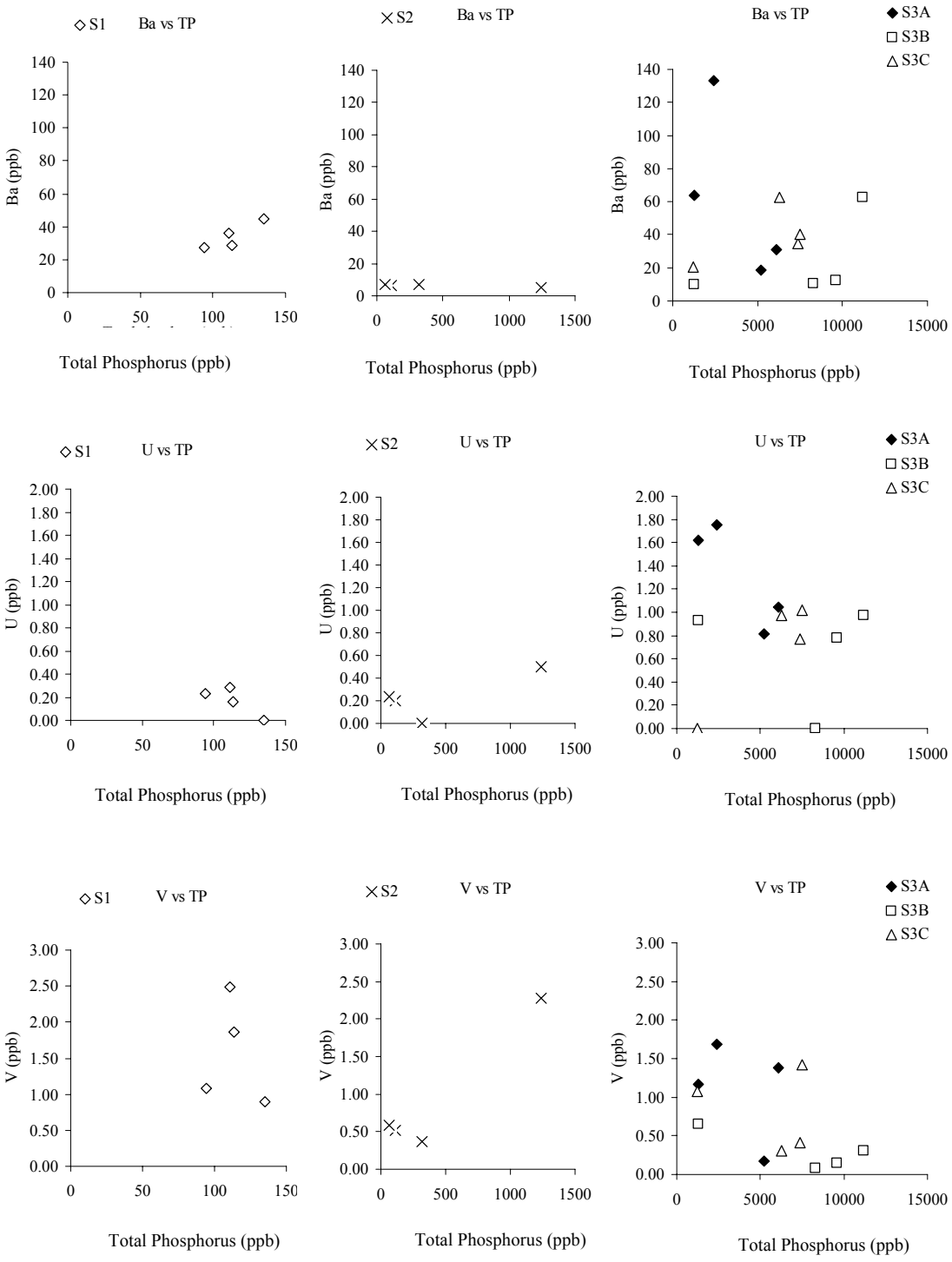

Figure C-1
Scatter Plots of Chemical Species Versus Corresponding Total Phosphorus for Five Source Sites (Cont.)

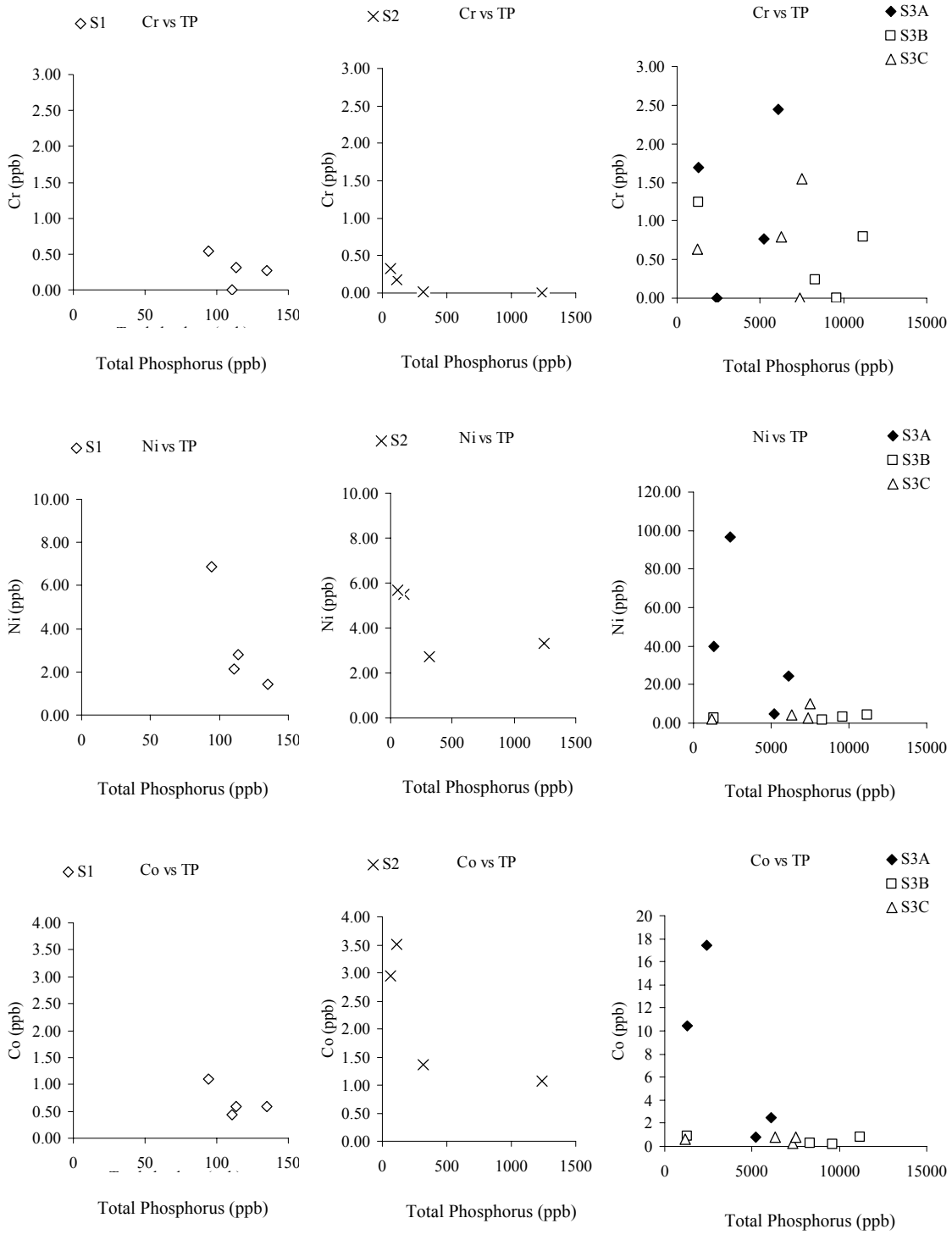

Figure C-1
Scatter Plots of Chemical Species Versus Corresponding Total Phosphorus for Five Source Sites (Cont.)

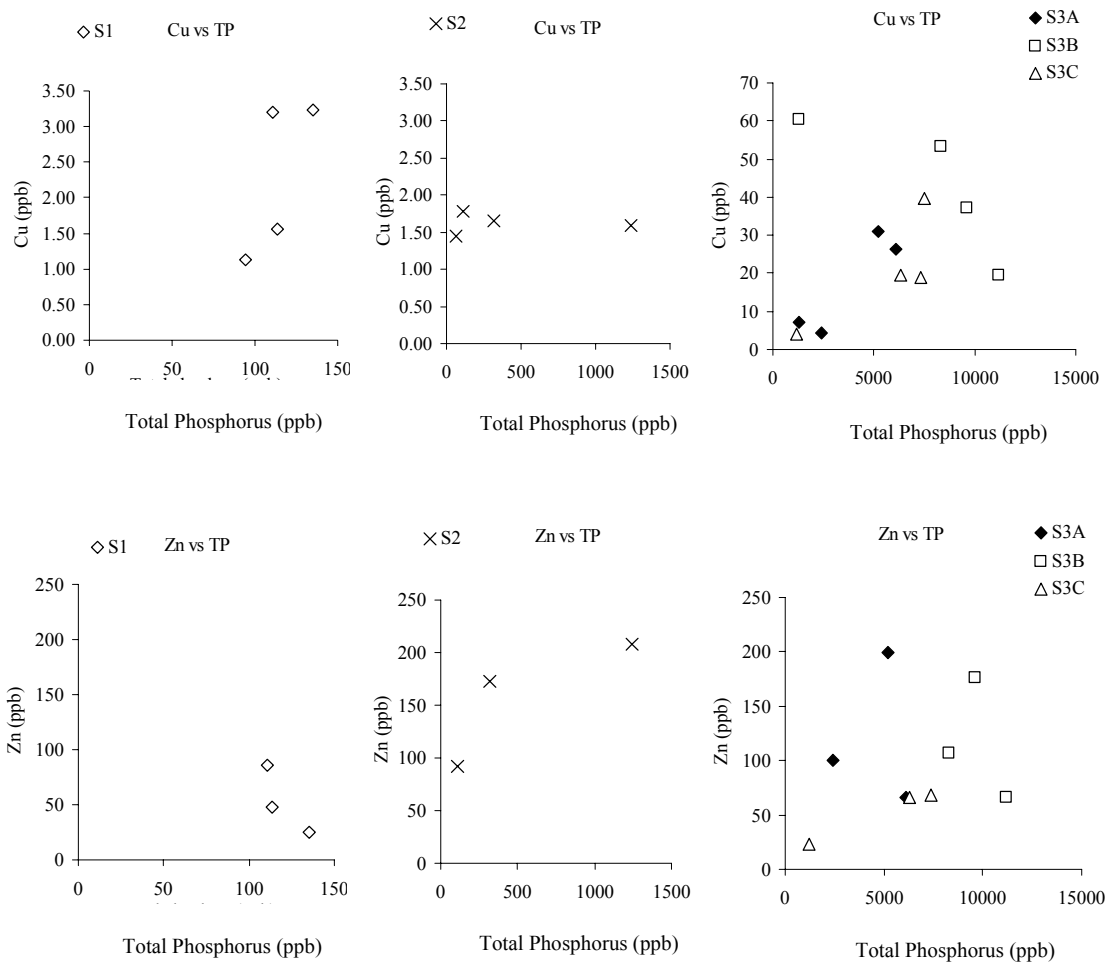

Figure C-1
Scatter Plots of Chemical Species Versus Corresponding Total Phosphorus for Five Source Sites (Cont.)

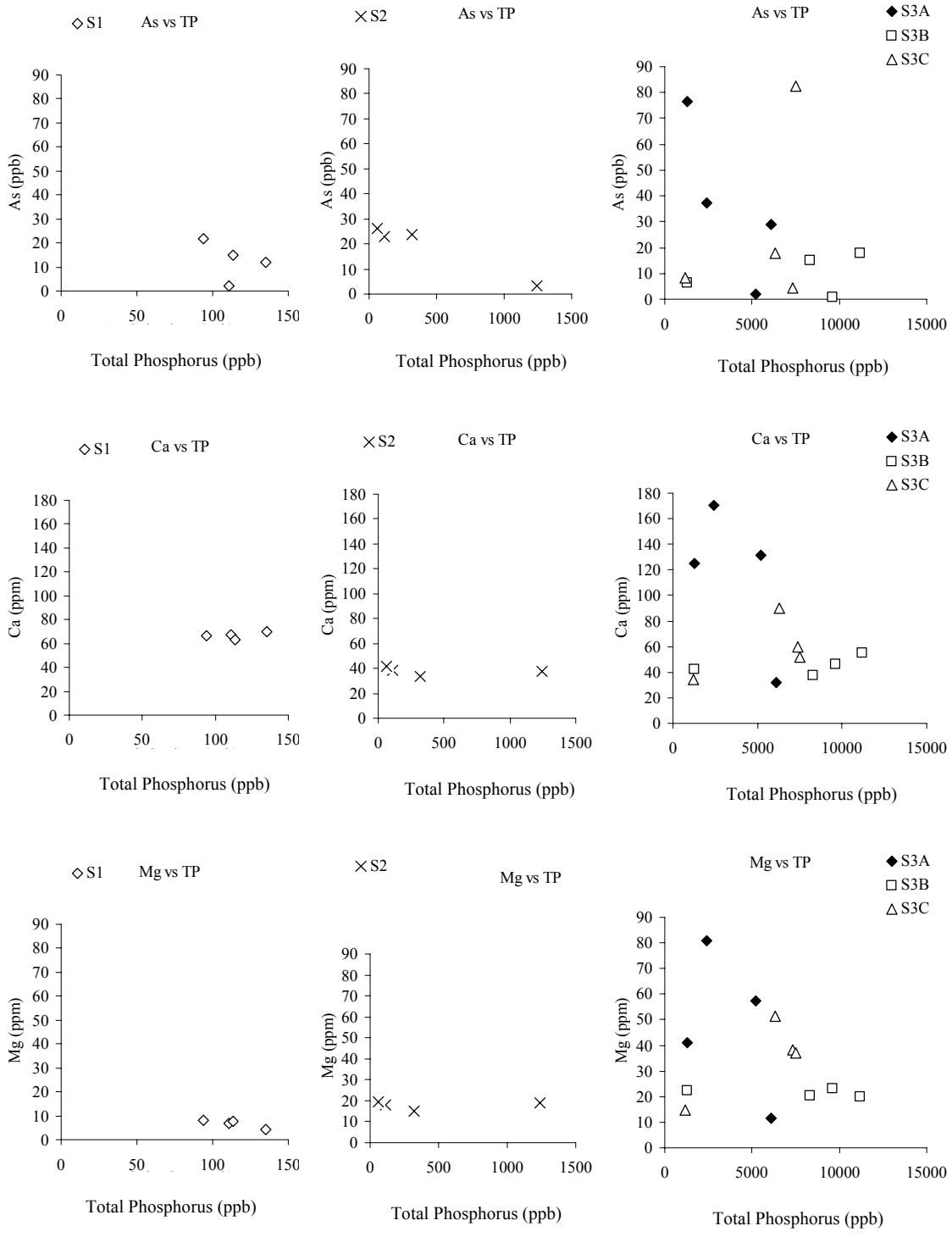

Figure C-1
Scatter Plots of Chemical Species Versus Corresponding Total Phosphorus for Five Source Sites (Cont.)

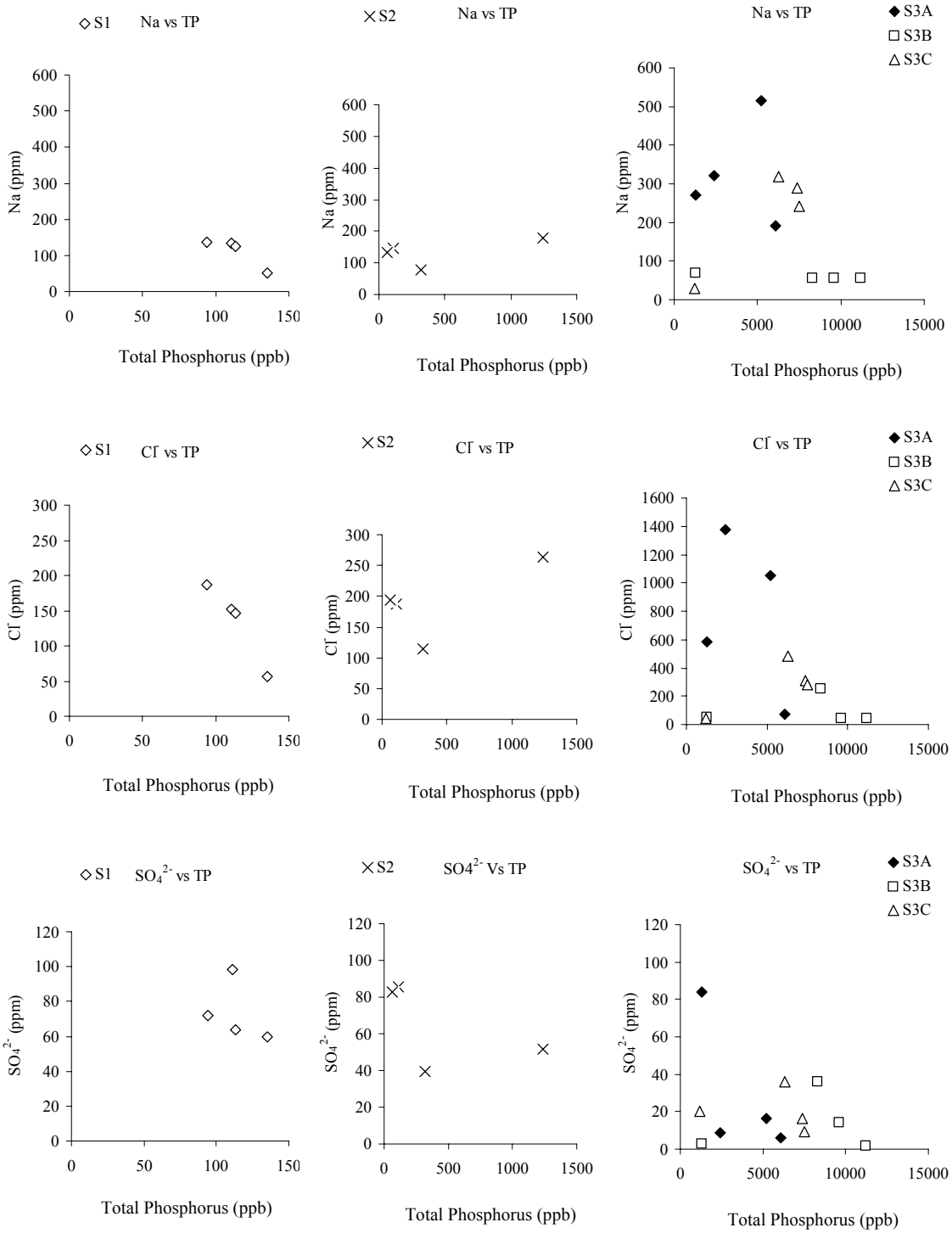

Figure C-1
Scatter Plots of Chemical Species Versus Corresponding Total Phosphorus for Five Source Sites (Cont.)

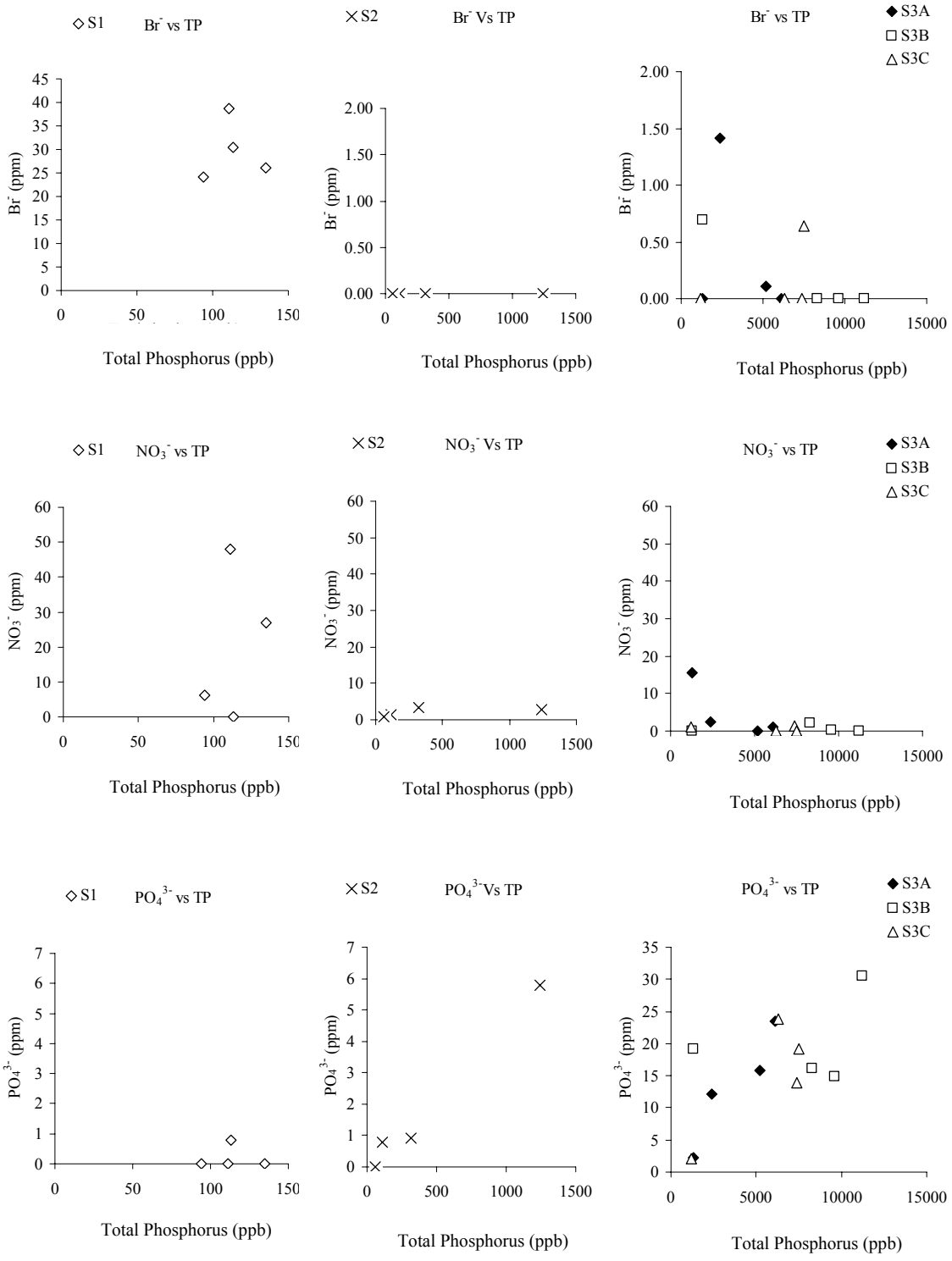

Figure C-1
Scatter Plots of Chemical Species Versus Corresponding Total Phosphorus for Five Source Sites (Cont.)

D FACTOR SCORES OF EACH SITE

Table D-1
Factor Scores of Each Site for PCA on All Sites

Site	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
S1, Apr 2004	-0.12	-0.09	5.91	-0.14	0.02	-0.05
S1, July 2004	-0.25	-0.32	2.26	0.45	1.10	1.45
S1, Oct 2004	0.07	-0.29	1.74	0.31	0.49	1.82
S1, Jan 2005	0.04	-0.01	2.85	0.15	-0.62	-0.08
A1, Apr 2004	0.22	-0.06	0.93	0.80	-0.44	-0.99
A1, July 2004	0.07	0.37	1.23	1.70	0.19	-1.29
A1, Oct 2004	0.03	-0.09	0.72	0.86	0.33	-0.29
A1, Jan 2005	-0.08	-0.20	0.28	0.40	-0.40	-0.57
C1, Apr 2004	0.17	-0.08	0.15	0.94	-0.41	-1.07
C1, July 2004	0.01	0.02	0.39	1.47	0.75	-1.16
C1, Oct 2004	0.17	0.16	0.53	1.43	0.34	-1.37
C1, Jan 2005	-0.11	-0.24	0.10	0.41	-0.41	-0.62
D1, Apr 2004	0.19	-0.22	-0.08	0.07	-0.44	-1.14
D1, July 2004	-0.09	-0.52	-0.55	-0.21	-0.14	-0.36
D1, Oct 2004	-0.33	-0.53	-0.62	-0.26	0.35	0.10
D1, Jan 2005	-0.22	-0.66	-0.71	-0.32	-0.33	0.16
S2, Apr 2004	0.00	0.30	0.27	0.54	-0.44	1.15
S2, July 2004	0.21	-0.64	-0.45	0.04	-0.44	3.04
S2, Oct 2004	0.15	-0.65	-0.44	0.07	-0.17	3.06
S2, Jan 2005	-0.08	-0.57	-0.55	-0.05	-0.24	1.82

Table D-1
Factor Scores of Each Site for PCA on all Sites (Cont.)

Site	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
A2, Apr 2004	-0.14	-0.38	-0.42	0.35	-0.49	-0.21
A2, July 2004	-0.15	-0.31	-0.64	0.65	-0.13	-0.42
A2, Oct 2004	-0.50	-0.48	-0.46	0.41	0.41	0.10
A2, Jan 2005	-0.24	-0.45	-0.63	0.34	-0.36	-0.04
C2, Apr 2004	-0.06	-0.37	-0.43	0.26	-0.66	-0.19
C2, July 2004	-0.11	-0.13	0.73	0.28	-0.46	-0.74
C2, Oct 2004	0.10	-0.07	-0.22	0.58	-0.46	-0.48
C2, Jan 2005	-0.08	-0.41	-0.45	0.16	-0.63	-0.14
D2, Apr 2004	-0.85	-0.68	-0.85	-0.23	-0.02	0.96
D2, July 2004	-0.31	-0.77	-0.26	-1.98	0.15	-0.41
D2, Oct 2004	-0.47	-0.89	0.09	-3.04	0.92	-0.71
D2, Jan 2005	-0.24	-0.72	-0.04	-0.96	-0.02	-0.33
S3C, Apr 2004	0.50	2.24	-0.19	-0.40	-1.51	-0.02
S3C, July 2004	0.88	3.16	-0.15	-0.11	-1.32	0.34
S3C, Oct 2004	-0.27	2.75	-0.52	0.22	1.82	1.78
S3C, Jan 2005	-0.38	-0.04	-0.44	0.31	0.36	0.40
S3A, Apr 2004	7.04	-0.95	-0.77	-0.06	1.17	-1.57
S3A, July 2004	1.67	2.82	-0.05	-1.45	-2.28	0.81
S3A, Oct 2004	2.50	-0.27	0.35	0.47	1.65	3.56
S3A, Jan 2005	-0.82	1.55	-0.58	-0.27	6.00	-0.86
S3B, Apr 2004	-0.40	1.90	-0.22	-0.98	-0.55	-0.51
S3B, July 2004	-0.38	3.77	-0.48	0.74	-0.13	-1.10
S3B, Oct 2004	-0.77	0.78	0.00	-1.76	1.71	-0.30
S3B, Jan 2005	-0.47	1.81	-0.06	-1.32	-0.32	0.43

Table D-1
Factor Scores of Each Site for PCA on all Sites (Cont.)

Site	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
A3, shallow, Apr 2004	-0.26	-0.47	-0.63	0.23	-0.45	0.01
A3, deep, Apr 2004	-0.26	-0.47	-0.63	0.23	-0.45	0.00
A3, shallow, July 2004	-0.38	-0.44	-0.69	0.38	-0.13	-0.01
A3, deep, July 2004	-0.35	-0.26	-0.66	0.41	-0.18	-0.10
A3, shallow, Oct 2004	-0.36	-0.37	-0.56	0.47	-0.06	-0.13
A3, deep, Oct 2004	-0.42	-0.44	-0.39	0.41	0.33	-0.11
A3, Jan 2005	-0.14	-0.40	-0.37	0.32	-0.70	0.43
D3, Apr 2004	0.16	-0.67	0.50	-2.88	-0.53	-0.64
D3, July 2004	0.09	-0.66	0.20	-2.76	-0.31	-0.55
D3, Oct 2004	0.00	-0.66	0.34	-3.23	0.33	-0.64
D3, Jan 2005	-0.70	-0.41	-0.26	-0.39	-0.43	1.02
A5, Apr 2004	-0.28	-0.46	-0.63	0.23	-0.40	-0.02
A5, shallow, July 2004	-0.34	-0.39	-0.30	0.43	0.17	-0.23
A5D, deep, July 2004	-0.28	-0.32	-0.39	0.45	-0.15	-0.32
A5, Oct 2004	-0.45	-0.29	-0.46	0.95	0.39	-0.40
A5, Jan 2005	-0.32	-0.50	-0.38	0.01	-0.49	-0.06
B, shallow, Apr 2004	-0.09	-0.29	-0.08	0.46	-0.48	-0.49
B, deep, Apr 2004	-0.11	-0.30	-0.07	0.46	-0.47	-0.48
B, shallow, July 2004	-0.34	-0.30	-0.51	0.71	-0.02	-0.31
B, deep, July 2004	-0.40	-0.35	-0.51	0.60	-0.02	-0.14
B, shallow, Oct 2004	-0.36	-0.38	-0.45	0.58	0.16	-0.12
B, deep, Oct 2004	-0.27	-0.43	-0.36	0.62	0.30	-0.18
B, Jan 2005	-0.24	-0.28	0.01	0.44	-0.33	-0.49

Table D-2
Factor Scores of Each Site for PCA on Source Sites

Site	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
S1, Apr 2004	-0.03	0.27	-0.62	-0.34	3.29
S1, July 2004	-0.48	0.57	0.17	0.05	1.27
S1, Oct 2004	-0.27	0.81	0.13	-0.18	0.69
S1, Jan 2005	-0.40	0.41	-0.95	-0.08	1.10
S2, Apr 2004	-0.45	0.76	-0.55	-0.09	-0.17
S2, July 2004	-0.37	1.55	0.01	-0.44	-1.04
S2, Oct 2004	-0.39	1.50	0.21	-0.42	-0.98
S2, Jan 2005	-0.74	1.01	-0.34	-0.09	-0.99
S3C, Apr 2004	0.50	-0.69	-1.00	-0.46	-0.43
S3C, July 2004	1.16	-0.55	-0.19	-0.80	-0.40
S3C, Oct 2004	0.29	-0.81	2.15	-0.54	-0.14
S3C, Jan 2005	-1.04	0.39	-0.45	0.31	-0.69
S3A, Apr 2004	2.14	0.34	-0.82	3.37	-0.24
S3A, July 2004	2.33	-1.01	-0.26	-1.68	-0.08
S3A, Oct 2004	1.28	1.30	2.34	0.00	0.08
S3A, Jan 2005	-1.38	-1.60	1.78	1.63	0.34
S3B, Apr 2004	-0.58	-1.09	-1.09	0.01	-0.53
S3B, July 2004	-0.50	-1.14	-0.59	0.36	-0.53
S3B, Oct 2004	-0.68	-1.07	0.48	-0.17	-0.07
S3B, Jan 2005	-0.39	-0.95	-0.41	-0.43	-0.46

Table D-3
Factor Scores of Each Site for PCA on All Sites Except Source Sites

Site	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
A1, Apr 2004	-0.38	1.65	-0.24	1.29	1.85	-0.45
A1, July 2004	0.69	2.27	-0.52	0.67	2.27	0.48
A1, Oct 2004	2.15	2.41	-0.37	1.25	0.59	1.96
A1, Jan 2005	-1.09	-0.17	0.02	0.04	0.37	1.57
C1, Apr 2004	-0.34	0.35	-0.51	-0.41	1.45	-0.75
C1, July 2004	2.59	0.00	-0.74	-0.34	0.91	1.03
C1, Oct 2004	1.38	0.07	-0.41	-0.49	1.72	1.03
C1, Jan 2005	-1.13	-0.49	-0.04	-0.24	0.24	1.27
D1, Apr 2004	-1.07	0.42	0.02	1.98	1.45	-1.64
D1, July 2004	0.52	-0.43	-0.06	-0.16	0.17	-1.37
D1, Oct 2004	1.63	-0.76	0.42	-0.13	-0.40	-0.63
D1, Jan 2005	-0.12	-0.49	-0.09	0.11	-0.49	-0.83
A2, Apr 2004	-0.65	-0.18	-0.30	-0.13	0.24	-0.44
A2, July 2004	0.32	-0.69	-0.70	-0.27	-0.20	0.38
A2, Oct 2004	1.19	-0.42	-0.47	-0.25	-0.67	-0.32
A2, Jan 2005	-0.46	-0.44	-0.61	-0.03	-0.60	0.32
C2, Apr 2004	-1.01	-0.26	-0.18	-0.26	0.67	-0.96
C2, July 2004	-1.77	-0.28	0.89	-0.63	0.79	2.61
C2, Oct 2004	-0.45	0.13	-0.03	-0.53	1.42	-0.62
C2, Jan 2005	-1.08	-0.37	-0.32	-0.03	0.07	0.02
D2, Apr 2004	0.12	0.86	-0.78	-0.22	-2.35	-1.41
D2, July 2004	0.32	-0.50	1.35	-0.30	-0.31	-0.96
D2, Oct 2004	1.80	-0.64	2.38	-0.14	-0.64	-0.44
D2, Jan 2005	-0.04	-1.65	0.57	5.85	-1.09	0.36

Table D-3
Factor Scores of Each Site for PCA on all Sites Except Source Sites (Cont.)

Site	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
A3, shallow, Apr 2004	-0.51	-0.06	-0.45	-0.15	-0.13	-1.08
A3, deep, Apr 2004	-0.52	-0.08	-0.44	-0.15	-0.13	-1.05
A3, shallow, July 2004	0.02	-0.28	-0.55	-0.35	-0.39	-0.72
A3, deep, July 2004	-0.01	-0.25	-0.53	-0.34	-0.24	-0.79
A3, shallow, Oct 2004	0.00	-0.62	-0.34	-0.46	-0.04	-0.66
A3, deep, Oct 2004	1.05	-0.66	-0.29	-0.25	-0.23	-0.62
A3, Jan 2005	-0.72	1.34	-0.66	-0.35	0.40	-1.23
D3, Apr 2004	-1.01	1.50	2.74	-0.23	-0.02	-0.39
D3, July 2004	-0.41	0.92	2.70	-0.45	-0.24	-0.23
D3, Oct 2004	0.95	0.42	3.75	-0.63	-0.26	0.20
D3, Jan 2005	-0.76	3.77	-0.99	0.05	-3.82	1.09
A5, Apr 2004	-0.73	-0.75	-0.20	-0.35	-0.28	0.24
A5, shallow, July 2004	0.61	-1.02	-0.11	-0.35	-0.44	1.11
A5D, deep, July 2004	-0.42	-1.03	-0.09	-0.46	-0.10	0.93
A5, Oct 2004	0.94	-0.93	-0.56	-0.56	-0.45	1.28
A5, Jan 2005	-1.28	-0.77	-0.08	-0.29	-0.61	0.67
B, shallow, Apr 2004	-0.87	-0.14	-0.20	-0.19	0.58	-0.25
B, deep, Apr 2004	-0.87	-0.13	-0.21	-0.16	0.54	-0.22
B, shallow, July 2004	0.19	-0.15	-0.78	-0.32	-0.32	-0.37
B, deep, July 2004	0.12	-0.13	-0.77	-0.26	-0.54	-0.24
B, shallow, Oct 2004	0.83	-0.24	-0.51	-0.16	-0.37	0.02
B, deep, Oct 2004	1.50	-0.13	-0.68	-0.05	-0.16	-0.25
B, Jan 2005	-1.21	-0.96	-0.05	-0.17	-0.22	2.37

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